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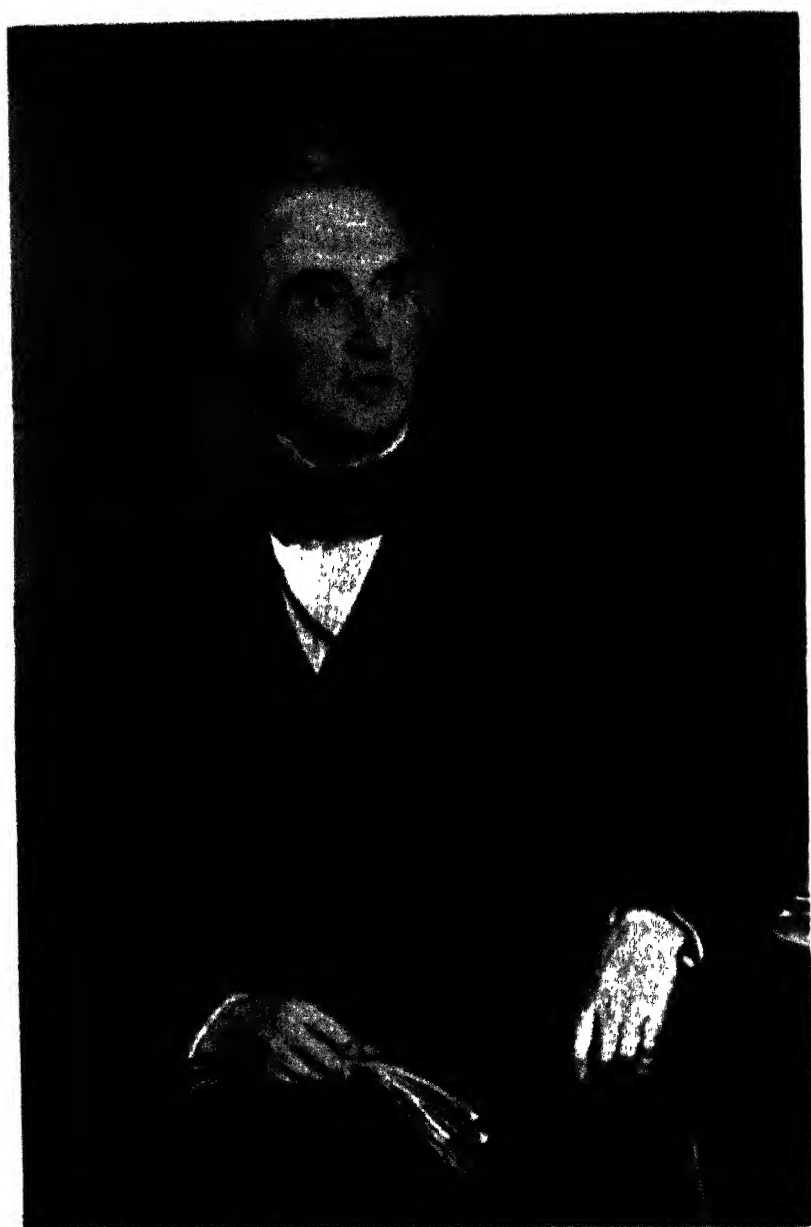
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JUSTUS VON LIEBIG

EDITORIAL REVIEW

JUSTUS VON LIEBIG

With the first number of the new volume the portrait of Lavoisier no longer graces the cover of the Journal; it has been replaced by that of Justus von Liebig, which one of his great-grandsons, Doctor Hesse, has permitted us to use for this purpose. The likeness has been prepared from a photograph of an oil painting by Trausehold which is still in the possession of the family.

Liebig was a chemist at a time when natural science and medicine had not yet broken loose from the fetters of speculation and philosophy. He introduced laboratory instruction, thereby giving to many for the first time a foundation for inventive experimentation. In consequence agricultural chemistry and physiology received a new guidance. Teaching concerning the nutrition of plants and animals owes to him a great deal in the way of new facts and of fruitful incentives by which we the living of to-day are often guided though quite unaware.

Liebig was born in 1803 at Darmstadt. His father was a dealer in dyestuffs. Many of these he himself prepared from directions in books on chemistry which he borrowed from the rich collection in the court library. Young Justus assisted him in the laboratory. This was, as he himself has written,¹ an excellent training for him. Here he laid the foundation for his art in experimentation; here he sharpened his powers of observation and his visual memory of many chemical processes. He made good use of his privileges in the court library.

¹ Ber. d. D. chem. Ges., 1890, Bd. 23, S. 817.

I read the books as they were placed there upon its shelves; from bottom to top, from right to left, it was all the same to me. I am certain that this manner of reading was of no special benefit to me in the acquisition of positive knowledge, but it did develop within me the incentive, which is proper to chemists even more than to other students of nature, of reflecting upon phenomena.

The profession of 'chemist' at that time did not exist. Therefore Liebig apprenticed to an apothecary, but was able to tolerate the situation for only a brief period. Thereafter he went to the university. But the chemistery taught there had nothing further to offer him. He went to Paris and worked with Gay-Lussac. There he became acquainted with Alex. von Humboldt, on whose warm recommendation he came in 1824 to Giessen as a second professor of chemistry, at first against the will of the faculty. It was therefore not made easy for him at the outset. But his ardor carried him through. He created the first teaching laboratory in Germany and thus made that small university for the time being the center of chemical study for the whole cultural world. His pupils became the most famous chemists of the time. His old laboratory still exists and is easily accessible, for Giessen is only an hour by railway from Frankfurt on the line to Berlin. No one should fail to visit this historic shrine and obtain for himself a living conception of the primitive apparatus with which Liebig and his pupils 100 years ago were able to obtain such beautiful results. Also his native city Darmstadt lies not far from Frankfurt, on the line to Basel, and possesses a Liebig museum well worth seeing.

In 1852 Liebig accepted a call to Munich. His health was impaired; consequently he relinquished the personal direction of the laboratory teaching. Authorship now engaged his time almost exclusively, and occupied the latter part of his life both richly and fruitfully. He died in 1873.

It would lead too far to picture all of Liebig's famous exploits in chemistry. Such a review also would be out of place here. But his accomplishments in physiology should be discussed and especially his influence on the doctrines of

nutrition. In this connection it is singular that none of the physiologists of that time was an immediate pupil of Liebig. Only a much later generation has recognized that chemistry is for the future one of its most indispensable aids, and that it cannot get along with physics and the microscope alone.

PLANT AND ANIMAL PROTEINS

We physiologists honor in Liebig the analyst who, with his own methods, first systematically investigated the organs of plants and animals as well as their feces, urine and bile. He established the protein content of legumes and cereals and called attention to the fact that they possess among themselves and with the albumins of the animal world almost the same elementary composition. In the course of this investigation he separated the nitrogenous from the non-nitrogenous organic foodstuffs and emphasized the fact that the same substances occur in our foods and as constituents of our bodies and that the latter arise from the former. The plastic (nitrogenous) foods together with water and minerals build up the body; from them proceed all phenomena of motion. The respiratory (non-nitrogenous) foods serve only for the production of heat. Liebig has often been misunderstood. Naturally he was also a child of his time and many of his expressions we should formulate somewhat differently and more clearly to-day. However, that does not gainsay the fact that Liebig was the first to envisage the relationship of foods correctly.

This conception of the differentness (*Verschiedenheit*) of the foodstuffs was both new and true and will endure, even in the light of newer conditions and relationships. Its simplicity and, one might say, its self evidence will never detract from the high honor of one who first expressed it, and indeed with entire clearness of knowledge of its significance.

These words of Bischoff uttered in 1874 in his memorable address on Liebig are as true to-day as they were at that time.

It was also Liebig, who in his famous and consequential Theory of Nutrition of Animals and Man, for the first time clearly worked out the simple circulation of the organic food-stuffs in nature. The first thought of this had come to him while in Paris. He himself writes concerning it:²

I recognized, or probably more correctly, it glimmered in my consciousness that not only was there a law of relationship governing all chemical phenomena in the mineral, vegetable and animal kingdoms, that none stands alone, but always is closely interlinked with another, and this one again with another, and so on, all bound together, but also that the origin and occurrence of things is like a wave motion moving in a circle.

LAW OF CONSERVATION OF ENERGY

With the help of the solar energy plants assimilate and make available their body substances to all other living beings for dissimilation. This view led Liebig in 1848,³ therefore before J. R. Mayer, Hemholtz and Joule, to the law of the conservation of energy and its validity in the animal body. In his earliest deductions he considered it unnecessary to throw overboard a peculiar life force; but it was for him only a principle of orientation in the organized world and not a particular form of energy as it appeared to his contemporaries. In the later editions of his 'chemical letters' he expressed himself more and more clearly. And this, although the numerically exact proof for the validity of the law in the animal body was only to become available much later. We marvel at the fruitful clearness of his ideas, in which he was far in advance of his contemporaries.

FAT FORMATION FROM PROTEIN

It is only natural that his views concerning processes of intermediary metabolism should differ essentially from those which are held probable to-day. Liebig at first sought in the

² Ber. d. D. chem. Ges., 1890, Bd. 23, S. 824.

³ The author must be in error regarding this date, as Mayer's contribution is dated 1842 and that of Helmholtz 1847.—Ed.

(plastic) proteins the mother substance for deposited fat; later he recognized that it must come in far greater part from starches. This appears to us now as a self-evident and well-established datum of physiology. At that time it was not so. It was still in Liebig's life time that Voit strove so earnestly to bring the earlier doctrine to victory, and fell into the well-known controversy with Pflüger. The latter denied the formation of fat from protein. Bischoff was not so positive; he came to his memorial address on Liebig in 1874⁴ to a *non liquet*. "However the final answer to the question, still actively discussed, may turn out, it will always remain an extraordinarily great service of Liebig to have brought it under investigation." The conflict of opinions was at that time maintained with a liveliness which to-day is not rightly understood. For the arguments and counter arguments which were adduced concerning fatty degeneration of organs, formation of adipocere, fermentative processes, inadequate respiration experiments, we do not regard to-day as any of them tenable. Fundamentally new material concerning the actual possibility of fat formation from protein has been brought forward only recently. But the question is not yet solved. It will be done only when we get an insight into the actual intermediary processes taking place, i.e., as, when and to how great an extent, probably persistent reductions in the strict sense are coupled with cleavages and oxidative metabolism.

MINERALS, LAW OF MINIMUM

Liebig's works on the mineral constituents of the organism have become best known. They constitute the point of departure for the 'law of minimum,' the significance of which does not need to be discussed more intimately here. Even to-day almost every issue of this Journal brings several contributions based upon this law. Originally it was deduced by Liebig from the ash analysis of different plants. That mineral constituent, which in relation to the need of the fruit is present

⁴Bischoff: Liebig's Einfluss auf die Entwicklung der Physiologie. München Akademie.

in the soil in the smallest quantity limits the plant's growth and determines the harvest yield. To-day we know that this law holds in general in the nutrition of every human being, and not for the minerals only, but for all constituents of the food, which cannot be prepared from other constituents. It holds for individual amino acid and led to the discovery of the vitamins; but it applies indeed also for definite kinds of sugar and fatty acids. It has occasioned one of the greatest branches of industry, the manufacture of artificial fertilizers, has led to the establishment of agricultural experiment stations, and, finally, it dominates even the commercial relations of foreign peoples one with another.

ACID-BASE ECONOMY OF SOILS AND OF LIVING BEINGS

Liebig found in the mineral constituents of the food the cause of the acid or alkaline reaction of the urine. The soils also show different reactions. One must seek plants adapted to a given soil. Its properties are changed in that definite minerals are withdrawn by the crops. Hence the necessity of rotation of crops and of fallow ploughing. From the views first promulgated and correctly developed with intuitive insight by Liebig have grown up the great doctrines of acid-base economy in both the living and the non-living worlds.

DIFFUSION

Liebig sought to find a basis for the differences in ash content of individual organs and thus came to a conception of disequilibria which are maintained in the life processes, and thence to the laws of diffusion. All this has for a long time become so generally accepted that we never reflect that all these conceptions are not yet 100 years old, all trace back to Liebig, and must have been won only by sagaciously devised experiments and by immensely painstaking analyses. Who knows to-day, for example, that it was Liebig who first made clear the detergent action of many neutral salts?

SOIL COLLOIDS

It is well known that the practical application of mineral fertilization was brought about by Liebig only after many years of the greatest disappointments and the severest hostility on the part of agriculture. For in the thought that the plants can take up only the dissolved minerals of the soil water and their impoverishment must be prevented, he with great pains transformed the phosphates into an insoluble form. Later he learned to recognize the great significance of the soil colloids and their powers of adsorption. I believe we as successors cannot be thankful enough that Liebig, through his obsession and his belief in the ideas of his time, was compelled to make this detour. Only by means of this has agricultural chemistry attained a secure foundation. And I am convinced that we animal physiologists shall still derive much of usefulness from these investigations. For *on* the colloid protoplasm, not in the tissue water, is where all intermediary processes take place. The identical laws, according to which the root hairs separate the soluble soil minerals from their adsorption compounds with the soil colloids and thus make them absorbable, are valid also for all those processes by which dissolved foodstuffs are taken up by the protoplasm of the cell, are directed to the proper places and then are transformed in an orderly but compelling fashion. Only thus can the life processes of the cell be maintained in an orderly progress.

FERMENTATION, CATALYSIS

In this field also, as it happens, Liebig not only advanced certain ideas but himself contributed very important research material in great abundance. His fundamental investigations on fermentation have become well known through his controversy with Berzelius and with Pasteur. They were not able, however, to lead him to an actual theory of catalysis. That could only come, as the history of every science shows, when quantitative measurements of the detailed processes which play a role in the 'drama' (Schönlein) of a catalytic reaction

are at hand. This became possible only after 1900 in an age in which the major industries were already making use of catalytic reactions. What a contrast between that day and this! Scarcely 100 years ago Liebig's small laboratory and his investigations on the nitrogen metabolism of plants, mineral fertilization, the fermentation of yeast; to-day the most imposing factories in which gas reactions are carried out with heterogeneous catalysis on the largest scale and ammonia and saltpeter become available to agriculture. Industry has extended Liebig's ideas and brought them to undreamed of fluorescence.

MEAT EXTRACTS

In an even wider field Liebig's discoveries and ideas are still to-day bringing forth fruit. His famous investigations on the composition of meat and his extractive substances have taught us to know creatin, creatinine and inosinic acid. Liebig was unable to gain a correct view of the physiological significance of these substances. Bischoff writes in this connection:⁵

Now it is actually possible to assume that none of these extractive substances are constituents of the living organs in the form in which we obtain them by chemical treatment of the organs. And so it seems to me the more remarkable that Liebig was correct in his opinion concerning the substances effective in the muscle in relation to its activity, since everything which has been concluded to the contrary from the experimentally demonstrated ineffectiveness of them (for example creatin) has fallen down.

Is this not proof of a magnificent foresight that a half century later the precursors of these compounds, creatine-phosphoric acid and adenylic acid, have come to be known and now for the first time we are beginning to get an insight into the role which those substances play in the mechanism of muscular activity.

This review has already become too long, for in the richness of significant discoveries which we owe to Liebig it is difficult to make the right choice. I believe, however, it has been

⁵Loc cit., p. 84.

shown that Liebig's portrait has been rightly chosen to appear on the cover page of this Journal in the immediate future. Many of his ideas concerning the interdependence of events in metabolism appear to us no longer convincing; that in the progress of our knowledge is not at all surprising. Many of his experimental findings indeed are no longer valid at all; that also is not to be wondered at. For the correctness of experimental findings depends absolutely and all together on the mode of procedure by which they are obtained, and in methodology very naturally we have made much progress in the decades which have elapsed since Liebig's death. This is true especially of his physiological studies and Liebig himself was perfectly aware that this would be the case.

It is a fundamental postulate for researches in physics and in chemistry, the inorganic sciences, never to leave the path from the known to the unknown and to proceed from the study of simple phenomena to those which are more complex. It is not always possible to proceed in this manner in the investigation of the life processes. What is there in this field that is known with equal certainty? Imagination is allowed a much wider range. Careful to the utmost degree in his analyses Liebig nevertheless possessed imagination and therefore he was able in the latter half of his life to make the applied sciences productive in so astonishing a manner which prevails even to this day. To these applied sciences belongs the science of nutrition.

I ascribe thus to Liebig a very great and very beneficent influence on the development of a better and more exact method of investigation in physiology and in medicine. And I stress these deserts of Liebig all the more because the effects of their influence will extend far beyond his own individual accomplishments. Yet it is just these influences which the present generation, and still more the future generations, will forget all too early to ascribe to him. Particularly those who grew up instructed in these better methods of investigation are very much inclined to think that conditions always have been as good. Besides, only a few have a liking for historical studies. The majority of people scarcely know anything about

the operations of science beyond the juncture of their own consciousness.⁶

This review ventures to do justice in this respect to the memory of Liebig.

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⁶ Bischoff, loc. cit., p. 61.

THE BIOLOGICAL VALUE OF RATIONS CONTAINING FISH MEAL¹

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ONE FIGURE

(Received for publication April 4, 1933)

This paper presents results showing the effect of adding varying amounts of protein in the form of Alaska herring fish meal to a basal ration fed to growing chicks. St. John, Carver, Helphrey, Miller and Cassel ('30) reported results obtained with dry skim milk as a protein supplement, but were unable to calculate the biological value of the protein of those rations, because a satisfactory method for determining the biological value of protein suitable for use with poultry was not available. St. John, Johnson, Carver and Moore ('32) designed a method for this purpose, and biological values for nitrogen are reported in the present paper. It would be highly desirable to use a so-called synthetic ration composed of purified food materials for this type of work, but, as shown in this laboratory (Helphrey, '29; Aspinall, '30) and by the work of others, such a ration has not been successfully used with poultry.

The general theory upon which the determination of biological value is based is discussed by Thomas ('09), Mitchell ('24, '26), Mitchell and Hamilton ('29) and others, and the reader is referred to these papers for this material.

¹Published as scientific paper no. 274, College of Agriculture and Agricultural Experiment Station, State College of Washington.

EXPERIMENTAL

Four lots of S. C. White Leghorn chicks were used, each lot consisting of thirty chicks which were placed in the nutritional batteries at the age of 48 hours. Each chick was weighed at the beginning of the experiment and once each week thereafter. A record of feed consumption and of the amount of excreta for each lot was kept and is reported for weekly periods. The method of caring for the chicks is described by St. John, Carver, Helphrey, Miller and Cassel ('30). The experiments were continued for 12 weeks beginning January 27, 1931. The excreta were collected, preserved and analyzed according to the methods described by St. John and Johnson ('31)² and St. John, Johnson, Carver and Moore ('32), except that, with the type of ration used in these experiments, it was found desirable to use 0.65 N hydrochloric acid for the preservation of the excreta. Swift, Black, Voris and Funk ('31) have recently emphasized the need for improved methods of experimental procedure in poultry work. Using the methods suggested by St. John and Johnson ('31) it has been possible to prepare a uniform sample for the determination of the uric acid, to make a quantitative determination of the uric acid, and to remove the feathers from the excreta after treating with hydrochloric acid and drying and before pulverizing. However, the number of feathers lost in the pens was reduced to a minimum by the method developed by Carver ('31). With the batteries and feed hoppers used (St. John, Carver, et al., '30, and Carver, St. John, et al., '32) there was practically no mixing of food and excreta.

The basal ration used consisted of: Ground yellow corn, 45 pounds; ground wheat, 25 pounds; wheat bran, 20 pounds; alfalfa leaf meal, 5 pounds; ground oyster shell, 3 pounds; bone meal, 1 pound; salt, 1 pound; fortified cod liver oil, $\frac{1}{4}$ pound. No scratch grain was fed at any time, the all-mash ration being fed exclusively. To this the Alaska herring meal was added to give the ratios shown in table 1. The per cent

² In this method, the paper used for filtering the alkaline solution after adding piperidine and heating was S & S no. 588.

of nitrogen in the fish meal was 10.66 ($N \times 6.25$ equals 66.6 per cent protein). The proximate analysis of the rations is shown in table 1.

TABLE 1
Composition of rations

LOT	RATIO BASAL RATION TO FISH MEAL	MOISTURE	ASH	ETHER EXTRACT	CRUDE FIBER	NITROGEN	PROTEIN
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
55	97: 3	11.2	5.8	4.9	5.3	2.10	13.1
56	93: 7	10.7	6.2	4.1	4.9	2.48	15.5
57	88: 12	10.8	6.3	4.0	4.8	3.10	19.4
58	80: 20	10.6	6.4	3.6	4.7	3.89	24.3

The growth curves are shown in figure 1, based upon the data shown in table 2.

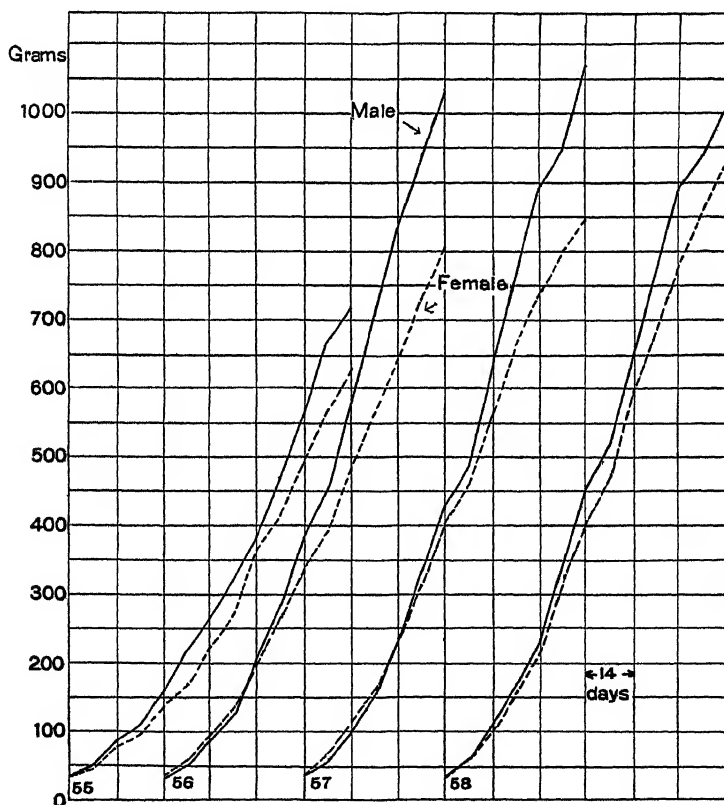


TABLE 2
Average weight of chicks by weeks

WEEKS	GM.	MALE		FEMALE		Gm.	MALE		FEMALE		
		No.	Gm.	No.	Gm.		No.	Gm.	No.	GM.	
Lot 55—13 per cent protein						Lot 56—15 per cent protein					
0	37.6	17	38.2	11	37.2	36.1	12	35.5	13	36.4	
1	50.1	17	51.3	11	49.2	52.5	12	50.8	13	53.8	
2	83.8	17	86.5	11	79.7	91.3	12	87.5	13	94.9	
3	105.3	17	109.9	11	98.2	131.2	12	127.5	13	134.6	
4	152.0	17	159.8	11	140.2	200.8	12	202.7	13	199.2	
5	197.5	16	213.1	9	169.8	277.5	12	287.7	13	268.2	
6	251.4	16	266.3	9	224.9	360.4	12	382.7	13	340.0	
7	303.2	16	319.8	8	270.0	421.4	12	455.0	13	390.5	
8	378.2	16	384.1	8	366.4	528.2	11	581.5	13	483.1	
9	452.8	16	468.4	8	421.8	628.6	11	704.7	13	564.2	
10	541.7	16	564.4	8	496.5	734.3	11	836.4	13	647.9	
11	634.3	16	666.8	8	569.5	830.7	11	945.6	13	733.7	
12	690.0	16	719.6	8	630.8	910.0	11	1034.7	13	804.6	
Lot 57—19 per cent protein						Lot 58—24 per cent protein					
0	37.7	17	37.7	13	37.7	38.4	16	38.8	13	38.2	
1	56.3	17	54.9	13	58.2	56.3	16	57.0	13	57.0	
2	104.4	17	100.6	13	109.5	109.0	16	111.7	13	105.8	
3	156.3	17	153.1	13	160.6	170.7	16	173.3	13	167.5	
4	234.7	16	234.4	13	235.3	232.5	16	238.3	13	225.6	
5	329.0	16	337.3	13	318.9	341.3	16	352.1	13	328.2	
6	419.8	16	429.8	13	407.7	432.6	16	452.0	13	408.9	
7	474.3	16	489.3	13	456.0	505.4	16	527.5	13	478.2	
8	608.1	15	646.9	13	563.4	629.4	14	656.0	13	600.9	
9	728.9	15	787.0	13	661.8	739.4	14	780.3	13	695.4	
10	820.4	15	891.2	13	738.8	837.6	14	889.1	13	780.0	
11	907.0	15	999.1	13	800.1	907.8	14	942.4	13	870.5	
12	966.3	15	1070.3	13	846.3	978.3	13	1017.7	13	938.9	

The curves for the two intermediate protein levels are approximately parallel, although the chicks fed the 19 per cent protein level had attained a slightly greater weight. Lot 55 with a protein level of 13 per cent showed distinctly less growth. The high level of protein produced a greater growth in the pullets, but less in the cockerels. The mortality in all lots was low as shown in table 2. The birds were thrifty throughout, with no disabling abnormalities. There was no evidence of nutritional leg paralysis such as that described by Bethke, Record and Kennard ('31) when fish meal was fed.

The average feed consumed per bird per week is shown in table 3.

TABLE 3
Average feed consumed per bird per week (grams)

WEEK	LOT 55	LOT 56	LOT 57	LOT 58
1	41.1	40.7	41.7	41.4
2	87.0	95.6	99.0	96.1
3	100.9	113.6	130.6	126.8
4	149.5	186.4	184.8	192.3
5	196.1	224.3	232.2	242.3
6	211.9	274.3	307.7	276.7
7	246.9	277.4	285.8	318.7
8	265.6	337.1	327.6	344.0
9	391.3	433.3	364.3	429.9
10	365.3	353.0	365.6	400.0
11	415.5	443.2	430.7	436.5
12	466.4	508.2	444.2	451.5

The only consistent variation in feed consumption between the four lots is found in the lower consumption by lot 55, which received the ration containing the lowest percentage of protein.

In table 4 is shown the amount of gain per unit of feed consumed by the chicks. In general the gain per unit of feed is somewhat larger for lots 56, 57, and 58 than for lot 55. This continues through the tenth week, but is not true for

TABLE 4
Grams gain per gram of feed

WEEK	LOT 55	LOT 56	LOT 57	LOT 58
1	0.27	0.34	0.44	0.43
2	0.37	0.34	0.49	0.53
3	0.21	0.35	0.40	0.49
4	0.31	0.37	0.40	0.32
5	0.14	0.36	0.41	0.45
6	0.25	0.29	0.30	0.33
7	0.17	0.22	0.19	0.23
8	0.28	0.26	0.36	0.25
9	0.19	0.23	0.33	0.26
10	0.24	0.30	0.25	0.24
11	0.22	0.22	0.20	0.16
12	0.12	0.16	0.13	0.08

each weekly period up to this age. For the eleventh and twelfth weeks the efficiency of the feed in producing growth decreases noticeably. Table 5, showing the gain per unit of

TABLE 5
Grams gain per gram of protein

WEEK	LOT 55	LOT 56	LOT 57	LOT 58
1	2.07	2.18	2.29	1.78
2	2.79	2.20	2.51	2.17
3	1.62	2.26	2.05	2.00
4	2.38	2.41	2.04	1.32
5	1.06	2.32	2.09	1.85
6	1.94	1.86	1.52	1.36
7	1.28	1.42	0.98	0.94
8	2.15	1.71	1.84	1.04
9	1.45	1.50	1.71	1.05
10	1.86	1.93	1.29	1.00
11	1.70	1.40	1.04	0.69
12	0.91	1.01	0.69	0.32

protein consumed, gives more definite information regarding the relative efficiency of different levels of protein. During the entire experimental period lot 56, receiving a ration containing 15 per cent protein, showed, on the average, the most efficient use of the total protein in the ration, while the low protein lot 55 was second. The high protein lot 58 was decidedly less efficient in its use of protein. During the first half of the period, lot 57 was somewhat more efficient than 55, but during the second half of the period the reverse was true; and in fact this latter was the most efficient of the four during this second half. The efficiency of protein utilization decreases with age, as was previously noted by St. John, Carver, Helphrey, Miller and Cassel ('30). This decrease is much more marked in the high protein lot 58 than in the other lots.

In tables 6 to 9 are given the data necessary for the calculation of the biological value of nitrogen. Detailed information on nitrogen intake and excretion are presented.

TABLE 6

Nitrogen intake and excretion at 13 per cent protein level (lot 55)

WEEKS	TOTAL DRY MATTER	URIC ACID	TOTAL NITROGEN	AMMONIA NITROGEN	URINARY NITROGEN	FECAL NITROGEN	TOTAL WEIGHT OF CHICKS	TOTAL FEED CONSUMED	TOTAL NITROGEN IN FEED CONSUMED	METABOLIC NITROGEN	ENDOGENOUS NITROGEN	BIOLOGICAL VALUE
	Gm.	Per cent	Per cent	Per cent	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
0							1130					
1	340.2	5.11	3.92	0.216	8.163	5.173	1454	1192	25.03	3.397	4.122	82.62
2	696.1	2.94	3.33	0.600	13.749	9.431	2347	2436	51.16	6.943	6.654	85.22
3	966.8	3.14	3.26	0.540	19.175	12.343	2948	2825	59.33	8.051	8.358	80.35
4	1478.6	4.43	3.20	0.124	29.585	17.730	4258	4188	87.95	11.936	12.071	78.68
5	1544.8	4.87	3.24	0.136	33.973	16.079	4938	4904	102.98	13.976	13.999	80.20
6	1744.4	3.71	3.42	0.440	36.559	23.099	6284	5299	111.28	15.102	17.815	81.85
7	1820.7	3.67	3.81	0.636	42.316	27.053	7276	5926	124.45	16.889	20.627	81.02
8	2030.0	1.57	3.54	1.104	41.294	30.568	9077	6375	133.88	18.169	25.733	87.19
9	2905.7	2.97	3.14	0.520	54.845	36.394	10868	9391	137.82	26.764	30.811	86.51
10	3203.5	2.26	3.14	0.640	55.794	44.796	13002	8768	175.36	24.989	36.861	87.83
11	3094.7	2.07	3.66	0.982	64.679	48.587	15224	9972	199.44	28.420	43.160	88.00
12	3256.5	2.53	3.90	1.092	78.780	48.224	16558	11193	223.86	31.900	46.942	84.66

TABLE 7

Nitrogen intake and excretion at 15 per cent protein level (lot 56)

WEEKS	TOTAL DRY MATTER	URIC ACID	TOTAL NITROGEN	AMMONIA NITROGEN	URINARY NITROGEN	FECAL NITROGEN	TOTAL WEIGHT OF CHICKS	TOTAL FEED CONSUMED	TOTAL NITROGEN IN FEED CONSUMED	METABOLIC NITROGEN	ENDOGENOUS NITROGEN	BIOLOGICAL VALUE
	Gm.	Per cent	Per cent	Per cent	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
0							1085					
1	343.7	4.78	4.14	0.216	7.773	6.456	1470	1142	28.32	3.255	4.167	85.64
2	671.1	3.25	3.73	0.500	13.283	11.749	2284	2391	59.30	6.814	6.475	87.48
3	987.8	4.68	3.74	0.240	22.226	14.718	3280	2840	70.43	8.094	9.299	79.74
4	1535.9	4.45	3.54	0.136	31.089	23.282	5021	4661	115.59	13.284	14.235	84.04
5	1860.4	4.87	3.54	0.208	42.588	23.270	7038	5608	139.08	15.983	19.952	82.82
6	2092.0	3.76	3.92	0.688	50.766	31.240	9012	6854	169.98	19.534	25.549	84.07
7	2103.8	3.43	4.26	1.036	57.310	32.312	10536	6934	171.96	19.762	29.870	82.79
8	2568.2	1.93	3.90	1.232	60.203	39.957	12676	8090	200.63	23.056	35.936	86.79
9	3119.6	3.94	3.52	0.480	69.931	39.879	15087	10400	239.20	29.640	42.772	88.14
10	3321.0	2.69	3.75	1.056	81.060	43.478	17623	8472	194.86	24.145	49.961	82.28
11	3046.8	1.93	4.18	1.540	83.153	44.203	19939	10638	244.67	30.318	56.527	88.46
12	3471.9	3.10	4.50	1.464	109.631	46.605	21842	12196	280.51	34.759	61.922	82.24

TABLE 8

Nitrogen intake and excretion at 19 per cent protein level (lot 57)

WEEKS	TOTAL DRY MATTER	URIC ACID	TOTAL NITROGEN	AMMONIA NITROGEN	URINARY NITROGEN	FECAL NITROGEN	TOTAL WEIGHT OF CHICKS	TOTAL FEED CONSUMED	TOTAL NITROGEN IN FEED CONSUMED	METABOLIC NITROGEN	ENDOGENOUS NITROGEN	BIOLOGICAL VALUE
	Gm.	Per cent	Per cent	Per cent	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
0							1132					
1	386.6	5.11	4.40	0.256	9.469	7.541	1689	1253	38.84	3.571	4.788	86.58
2	910.7	5.26	4.46	0.420	24.741	15.876	3134	2970	92.07	8.465	8.885	81.27
3	1386.7	6.33	4.22	0.192	39.901	18.618	4690	3919	121.49	11.169	13.296	76.67
4	1888.7	6.17	4.46	0.239	54.193	30.038	6809	5360	166.16	15.276	19.304	76.95
5	2457.8	6.76	4.74	0.376	80.779	35.721	9542	6735	208.79	19.195	27.052	72.06
6	2565.2	5.73	5.12	0.940	91.385	39.953	12176	8923	276.61	25.431	34.519	78.30
7	2679.5	6.06	5.46	1.016	101.688	44.613	13756	8289	256.96	23.624	38.998	73.43
8	2919.8	3.66	5.10	1.672	105.550	43.360	17028	9174	284.40	26.146	48.274	78.56
9	3690.0	6.42	4.64	0.496	121.585	49.631	20410	10202	299.94	29.076	57.862	77.19
10	3906.7	4.12	4.85	1.352	133.089	56.386	22972	10238	301.00	29.178	65.126	75.18
11	3434.5	3.73	5.29	1.912	135.463	46.222	25396	12061	354.60	34.374	71.998	81.48
12	3686.8	4.41	5.42	1.744	148.118	51.707	27056	12438	365.68	35.448	76.704	79.56

TABLE 9

Nitrogen intake and excretion at 24 per cent protein level (lot 58)

WEEKS	TOTAL DRY MATTER	URIC ACID	TOTAL NITROGEN	AMMONIA NITROGEN	URINARY NITROGEN	FECAL NITROGEN	TOTAL WEIGHT OF CHICKS	TOTAL FEED CONSUMED	TOTAL NITROGEN IN FEED CONSUMED	METABOLIC NITROGEN	ENDOGENOUS NITROGEN	BIOLOGICAL VALUE
	Gm.	Per cent	Per cent	Per cent	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
0							1152					
1	364.1	7.15	5.66	0.288	12.159	8.449	1689	1243	48.35	3.543	4.788	83.03
2	838.9	8.58	5.98	0.448	34.689	15.477	3163	2789	108.50	7.949	8.967	74.53
3	1269.9	9.25	5.82	0.272	53.261	20.647	4950	3677	143.04	10.479	14.033	70.48
4	1950.1	9.40	5.89	0.272	83.009	31.852	6745	5578	216.98	15.897	19.122	68.22
5	2202.2	9.95	6.29	0.448	103.633	34.885	9899	7027	273.35	20.027	28.064	70.77
6	2719.4	9.63	6.55	0.788	135.903	42.218	12548	8027	312.25	22.877	35.574	65.75
7	2877.0	10.91	7.03	0.992	166.459	35.749	14656	9244	359.60	26.345	41.550	64.33
8	2941.9	5.46	6.60	2.416	155.774	38.391	16996	9290	361.38	26.477	48.184	69.21
9	3646.9	10.10	6.23	0.808	190.308	36.894	19964	11608	427.18	33.083	56.598	68.42
10	4018.7	9.24	6.73	1.460	228.061	41.985	22588	10802	397.52	30.786	64.037	57.54
11	3409.4	8.74	7.46	2.380	225.589	28.752	24510	11786	433.73	33.590	69.486	64.41
12	3587.0	9.59	7.31	1.976	231.929	30.281	25436	11740	432.04	33.459	72.111	63.28

The amount of dry matter excreted increases as the per cent of nitrogen in the ration increases up to the highest level where there is a decrease. There is also a marked increase in the per cent of total nitrogen in the excreta as the level of nitrogen in the feed increases. In fact, this amount of nitrogen in the excreta of birds receiving a feed containing 24 per cent protein is from one and one-half to two times as much as in the excreta of those receiving only 13 per cent protein in the feed. The per cent of uric acid in the excreta is approximately the same for lots 55 and 56, but is higher for lot 57, and is markedly higher for lot 58. The per cent of uric acid in the excreta of the latter lot is in most cases from two to four times that in lot 55, the low protein lot. The per cent of ammonia nitrogen fluctuates, but shows little consistent variation with protein levels, except in the latter part of the experimental period, when it is somewhat higher in the higher protein lots. The per cent of ammonia nitrogen in the excreta using these rations was materially higher than that found by St. John, Johnson, Carver and Moore ('32) in the excreta when a low nitrogen diet was fed.

It appears that the urinary nitrogen may be expected to increase with an increase in the protein level in the feed. Inspection of tables 6 to 9 shows that in the early part of the experimental period the amount of urinary nitrogen eliminated by lot 58 is approximately two and one-half times as great as the amount eliminated by lot 55. The difference increases, until at the tenth week there is four times as much eliminated by the high as by the low protein lot. At this point lot 58 seems to have reached a maximum, while the other three lots continue to increase throughout the 12-week period. Through the 12 weeks lots 56 and 57 are intermediate between the other two, showing a progressive increase from low to high protein feeding levels.

The data for fecal nitrogen show a variation quite different from the urinary nitrogen. There is some increase in the fecal nitrogen with increased nitrogen feeding level during the first half of the experimental period. During the latter

half of the period the fecal nitrogen is less in lot 58. During the last 2 weeks it is approximately the same in the first three lots. It may also be noted here that the amount of urinary nitrogen is materially higher than the fecal nitrogen. The same was found by St. John, Johnson, Carver and Moore ('32) using a low nitrogen diet.

From the data recorded in tables 6 to 9, the biological value of the rations containing varying levels of protein were calculated each week by the method proposed by St. John, Johnson, Carver and Moore ('32). A summary of these values is presented in table 10. The biological values are based on group averages. Such a procedure was recommended by Ackerson, Blish and Musschl ('23) as a result of the study of individual variation. Swift, Black, Voris and Funk ('31), in a preliminary paper, believe that individual birds in separate cages should be used. Ackerson, Blish and Mussehl ('30) present evidence to support their contention that biological values should be based on group averages.

TABLE 10

The biological values of rations containing Alaska herring fish meal at various nitrogen levels

WEEKLY PERIOD	LOT 55 13 PER CENT PROTEIN		LOT 56 15 PER CENT PROTEIN		LOT 57 19 PER CENT PROTEIN		LOT 58 24 PER CENT PROTEIN	
	A ¹	B ²	A	B	A	B	A	B
1	82.62	81.30	85.64	84.73	86.58	85.92	83.03	82.41
2	85.22	84.04	87.48	86.42	81.27	80.12	75.53	73.40
3	80.35	78.58	79.74	78.26	76.67	75.43	70.48	69.44
4	78.68	76.71	84.04	82.84	76.95	75.78	68.22	66.89
5	80.20	78.44	82.82	81.77	72.06	70.81	70.77	69.77
6	81.85	80.36	84.07	83.12	78.30	77.32	65.75	64.76
7	81.02	79.48	82.79	81.94	73.43	72.57	64.33	63.36
8	87.19	86.34	86.79	86.18	78.56	78.03	69.21	68.59
9	86.51	85.33	88.14	87.45	77.19	76.75	68.42	67.68
10	87.33	87.02	82.28	81.91	75.18	75.00	57.54	57.02
11	88.00	87.24	88.46	88.11	81.48	81.25	64.41	64.03
12	84.66	83.69	82.24	81.61	79.56	79.38	63.28	62.95
Average	83.68	82.38	84.54	83.70	78.10	77.36	68.33	67.52

¹ Based on metabolic nitrogen per kilogram of feed.

² Based on metabolic nitrogen per kilogram of body weight.

Column A in table 10 gives the biological values calculated on the basis of a constant metabolic nitrogen value per kilogram of feed consumed. The biological values based on the metabolic nitrogen per kilogram of body weight are also given in column B. It is apparent that the values in column A are higher, although the difference between the two is slight. It is apparent that the biological value is higher for lots 55 and 56. This is especially evident if these data are plotted. This is apparent both from a comparison of the values week by week and from the averages. Lots 57 and 58 show an appreciably lower biological value. It should also be noted that lot 58, which received the ration containing the highest per cent of protein, shows a decrease in biological value with increasing age of the chicks. There is variation from week to week in all lots, but a general decrease with advancing age in lot 58. The level of the biological value remains about the same throughout the experimental period for the other three lots.

Preliminary to the work just discussed a determination of the biological value of a basal ration similar to but not identical with the ration used in this work was made and is reported by Gerritz ('31). The basal ration used contained no animal protein supplement, and the per cent of protein was 11.4. The growth on this ration was below normal, the chicks placed on it at the age of 2 weeks having attained a weight of only 475 gm. at 11 weeks of age, in comparison with an average weight of 800 or 900 gm. at that age. However, the chicks seemed in good condition. The growth curve of a second lot, transferred from a commercial ration at 11 weeks of age, did not show the normal slope up to 15 weeks of age. The biological value of the basal ration each week between the age of 3 and 11 weeks was as follows: 65.75, 77.33, 78.47, 79.71, 80.15, 75.68, 70.63, 76.19, 56.05. The second lot started at 11 weeks of age, showed weekly biological values of 70.80, 75.85, 77.03, 81.98. With the younger group there was an increase up to 7 weeks of age, followed by a decline. The older group showed increasing values for the 5-week period. It is evident that the biological value of the basal ration used is materially

below that of the ration carrying the herring meal supplement when used in quantities sufficient to make the protein level 12 and 15 per cent. However, it is above the value obtained when the protein level was 23 per cent.

Bringing together the different factors discussed, it is apparent that the two high protein rations fed to lots 57 and 58 produce slightly better growth than that fed lot 56, and all three are markedly better than the low protein ration. The chicks on the latter ration consume less feed than the other three lots, which are about equal. The gain per gram of feed consumed is about equal for the three high protein lots, but less for the low protein lot. The gain per gram of protein consumed is greater for lot 56, the ration containing 15 per cent protein, although it is not strikingly above lots 55 and 57. Lot 58 shows decidedly less gain per gram of protein consumed. The biological value of the rations for lots 55 and 56 is approximately the same and distinctly above that for the two high protein lots. The biological value of the two low protein rations is also above that of the basal ration, while the high protein ration is below the basal ration. These facts support the conclusion that a protein level of about 15 per cent shows optimum efficiency for this ration when fed to growing chicks. Whether or not the somewhat increased growth obtained with higher levels of protein is desirable is still open to question. The study of this question is being continued. The value of fish meal for older chicks is being studied and will be reported later. The digestibility of protein by chicks was not influenced to any marked extent by the level of protein intake as was shown by Swift, Black, Voris, and Funk ('31) using a protein supplement composed of 75 per cent dried buttermilk, 12.5 per cent fish meal, and 12.5 per cent meat scrap.

The efficiency of the fish meal in promoting growth seems to be high, as shown by the gain per gram of protein and the gain in bird weight. The gain per gram of protein consumed is approximately equal to that for dry skim milk at the different levels, as reported by St. John, Carver, et al. ('30),

and Carver, St. John, et al. ('32) and is above that for meat scrap as reported by Norris and Heuser ('30). Growth was also above that reported by Swift, Black, Voris and Funk ('31) using a mixed supplement of buttermilk, fish meal and meat scrap. The weight of the chicks at 12 weeks was above that of the chicks fed dry milk and was from 200 to 300 gm. above that of the chicks fed meat scrap.

SUMMARY

The results of a study of the supplementary value of herring fish meal fed at different levels in an all-mash ration for growing chicks are given. The biological value of these rations is high. It decreases somewhat as the level of total protein increases. The chicks receiving the ration containing 15 per cent total protein showed a slightly more efficient use of protein based on the gain per gram of protein consumed. Increased percentage of protein was accompanied by increased rate of growth.

Based on growth, feed utilization, protein utilization, and biological value, the 15 per cent protein level in this all-mash ration appears to be more efficient under the conditions used than the other protein levels used.

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THE VITAMIN B AND VITAMIN G CONTENT OF BOSCO PEARS. USE OF THE MUNSELL METHOD OF ASSAYING FOODS FOR VITAMIN G

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TWO FIGURES

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The Bosc pear is a variety particularly adapted to growth in Oregon and is produced abundantly in the southern part of the state. Its production is being studied at the Oregon Agricultural Experiment Station and extensive work is being done to establish markets in the East for the fruit. A mineral analysis of the Bosc pear has been completed in the department of chemistry and a Purnell research project has been conducted in the department of foods and nutrition to determine the most satisfactory methods of baking and serving Bosc pear (Kolshorn, '31). A study of the vitamin content seemed worthwhile, since no such studies have been made on this variety of pear.

The vitamin A and vitamin B content of Bartlett pears, raw and canned, reported by Kramer, Eddy and Kohman ('29) indicated that 5 to 7 gm. per rat per day was sufficient to maintain life without significant growth over a 60-day period, but there was a vitamin deficiency manifest in all the animals receiving these amounts. Neither in this investigation nor any other subsequent one have the components of the vitamin B complex been determined in pears. The studies here reported deal not only with the vitamin B complex, but with the content of vitamins B and G of Bosc pears.

EXPERIMENTAL

Preparation of the pears

The Bosc pears used in these experiments were harvested, ripened and stored under carefully controlled conditions, in accordance with the procedure now used for market practices. The fruit was grown at the College Experiment Station at Medford, Oregon, harvested and stored at a temperature of 32°F. The pears were ripened in 6 days at a temperature of 60° to 68°F. in a special ripening chamber, then held for 2 days in storage at a temperature of 32°F. when they were placed in a tunnel recirculation prune drier with a dry air circulation draft of about 600 feet per minute. Drying was accomplished in approximately 64 hours at a temperature averaging 160°F. (never in excess of 165°F.). After drying they were tightly sealed in no. 2½ cans and opened only for immediate use. The moisture content taken from sufficient samples of the dried product was found to be 9.67 per cent. The average moisture content of the fresh fruit was 80.17 per cent. Accumulative evidence that vitamin B is unaffected by drying led to this choice of procedure in preparing the pear for feeding tests (Sherman and Grose, '23).

Method. Healthy, normally growing albino and pied rats were weaned at 21 days and placed upon the experimental diets at 28 days when the average weight was about 40 gm. With the exception of two litters on Steenbock's ('23) stock ration, the diets used in the colony were either Sherman's ('22) diet 13 or Smith and Bing's ('28) modification of this diet.

The animals were distributed on the various experimental diets with weight, sex, and ancestry considered, in order that any influence due to these factors might be equalized in each experimental group. From five to twelve animals were used in each series.

The basal ration was a modification of the Sherman and Spohn ('23) diet 107 and had the following composition: purified casein, 18 per cent; dextrinized cornstarch, 66 per

cent; butter fat, 8 per cent; Osborne and Mendel ('19) salt mixture, 4 per cent; cod liver oil, 2 per cent; and agar, 2 per cent. Similar diets have been shown to be adequate in every respect, except for vitamin B complex.

The casein was purified by the leaching method (Steenbock et al., '23) and a preliminary test showed this product to be comparable in purity to the B- and G-free casein obtained from the Harris Laboratories.¹ The cornstarch was dextrinized by a method described by Palmer and Kennedy ('27). The butter fat and the cod liver oil were incorporated in the basal ration which was stored in a refrigerator for daily use.

TABLE 1

Effects of feeding Bosch pear as a source of undifferentiated vitamin B

DIETS	NUMBER OF RATS	AVERAGE INITIAL WEIGHT (GRAMS)	LENGTH OF DEPLETION PERIOD (DAYS)	AVERAGE WEIGHT AT END OF DEPLETION (GRAMS)	AVERAGE GAIN OR LOSS PER WEEK (GRAMS)	AVERAGE NET GAIN OR LOSS PER GRAM OF FOOD PER WEEK (GRAMS)	PER CENT POLYNEURITIS
Basal diet alone (vitamin B complex-free)	7	48	- 2.0	- 0.15	..
Basal diet + 0.5 gm. dried whole yeast	6	38	14.5	43.5	+ 13.3	+ 0.24	..
Basal diet + 1.0 gm. dried Bosch pear	8	41	14.0	44.5	+ 1.3	+ 0.02	87
Basal diet + 1.5 gm. dried Bosch pear	8	41	14.0	44.5	+ 4.2	+ 0.14	..
Basal diet + 1.75 gm. dried Bosch pear	5	43.2	14.2	47.4	+ 4.8	+ 0.20	..

Vitamin B complex in Bosch pears

All animals were 'depleted' of body storage of vitamin B complex by placing them on the basal ration until a stationary weight or a slight loss of weight was noted. The average time of this depletion period was 14 days (table 1).

Preliminary tests indicated the use of at least three levels of dried pear for vitamin B complex determinations, namely,

¹ The Harris Laboratories Inc., Tuckahoe, New York.

1.0 gm., 1.5 gm. and 1.75 gm., equivalent to 8.29 gm., 12.44 gm. and 14.57 gm. of fresh pear, respectively. Normal growth was obtained when the basal ration was supplemented by 0.5 gm. of dried yeast² daily (table 1), thus serving as a positive control while the basal ration alone produced the usual decline and death and thus served as a negative control.

Results. When 1.0 gm. of dried Bosc pear was fed daily as a source of the vitamin B complex, polyneuritic symptoms were noted with little exception and the average gain per week through the 8 weeks' experimental period was only 1.31 gm. while the gain per week on the basal diet supplemented by 0.5 gm. of dried yeast furnishing the vitamin B complex was 13.3 gm. A progressively better growth resulted on the higher levels of dried pear (table 1). No tests were made to discover what amount of dried pear would furnish sufficient quantities of the vitamin B complex to produce normal growth.

Expressing the results in Sherman units ('32), this limited number of tests indicates that 1.21 gm. of dried pear (10.03 gm. of fresh pear) furnish one unit of vitamin B complex.

Vitamin B (B₁) in Bosc pears

To determine the vitamin B (B₁) in Bosc pears the same basal ration was used supplemented by autoclaved yeast as a source of vitamin G (B₂). Methods and results vary considerably regarding the time and temperature used in autoclaving to destroy vitamin B (B₁) and retain the maximum quantity of vitamin G. It is now well known that the hydrogen ion concentration influences the retention of the vitamin G factor. Chick and Roscoe ('30) found that yeast at the normal hydrogen ion concentration autoclaved for 5 hours at 120°C. contained only 50 per cent of the vitamin G present originally and was apparently free of vitamin B. The yeast used in the present investigation was autoclaved for 5 hours at 120°C. and when fed at pH 5 at a level of 0.5 gm. daily it furnished enough vitamin G for normal growth when vitamin B (B₁) was supplied.

² Obtained from Standard Brands Co., New York City.

Supplements of dried pear were fed at three different levels 1.0 gm., 1.5 gm., 1.75 gm., respectively, as a source of vitamin B (B_1).

Results. The results are shown in table 2 and chart 1. That the autoclaving of the yeast was effective in destroying vitamin B (B_1) was demonstrated, since animals on the basal diet plus 0.5 gm. autoclaved yeast declined and death occurred

TABLE 2
Effects of feeding Bosch pear as a source of vitamin B (B_1)

DIETS	NUMBER OF RATS	AVERAGE INITIAL WEIGHT (GRAMS)	LENGTH OF DEPLETION PERIOD (DAYS)	AVERAGE WEIGHT AT END OF DEPLETION (GRAMS)	AVERAGE GAIN OR LOSS PER WEEK (GRAMS)	AVERAGE NET GAIN OR LOSS PER GRAM OF FOOD PER WEEK (GRAMS)	PER CENT POLYNEURITIC
Basal diet + 0.5 gm. autoclaved yeast (vitamin B (B_1) free	10	38	14.2	42.5	- 1.6	- 0.05	20
Basal diet + 0.5 gm. autoclaved yeast + 1.0 gm. dried Bosch pear	10	37.3	14.5	44.8	+ 3.0	+ 0.12	80
Basal diet + 0.5 gm. autoclaved yeast + 1.5 gm. dried Bosch pear	12	42.0	15.2	46.6	+ 5.5	+ 0.21	..
Basal diet + 0.5 gm. autoclaved yeast + 1.75 gm. dried Bosch pear	5	44.2	14.4	44.8	+ 8.3	+ 0.24	..

at the same time as animals fed the basal diet alone. When 1.0 gm. of dried Bosch pear plus 0.5 gm. autoclaved yeast were fed as supplements to the basal diet, an average gain of 3.0 gm. per week was obtained. While this amount of pear produced a small gain in weight, it did not prevent the occurrence of polyneuritic symptoms in any except two animals in this group. The animals showed weakness and head retraction and severe convulsions occurred in four animals. Autopsy

findings gave evidence of a vitamin B deficiency. That 80 per cent of the animals developed polyneuritis on the 1.0 gm. level of pear, while only 20 per cent demonstrated these

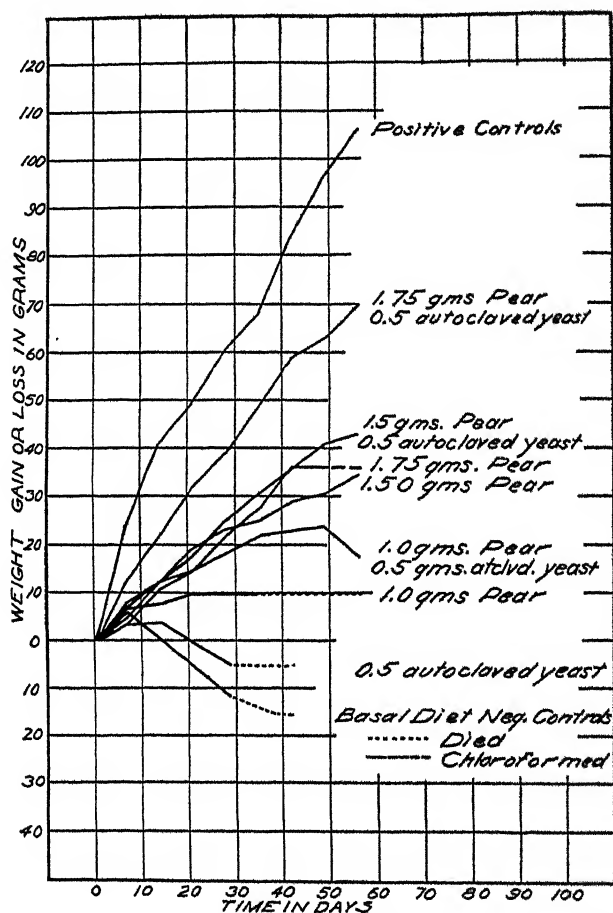


Chart 1 Results on growth of different amounts of dried Bosc pear as sources of vitamin B complex and vitamin B.

symptoms when no pear was fed is a fact concordant with the observations in similar investigations. Death often occurs too quickly on a diet entirely devoid of vitamin B for the animal to show symptoms characteristic of this deficiency.

With 1.5 gm. of dried pear as a supplement and 0.5 gm. of autoclaved yeast, there was an average weekly gain of 5.5 gm. and no symptoms of polyneuritis were manifested. Autopsy showed normal organs and no curvature of the spine.

The average weekly gain on the 1.75 gm. supplement of dried pear was 8.3 gm. Some difficulty was experienced at first in inducing the animals to consume this daily portion of pear. However, by removing the basal diet until the pear was consumed, the difficulty was eliminated. The animals in this group compared favorably with the positive controls (dried yeast supplement) in appearance, but failed to make more than 62 per cent of the gain made by the controls.

A progressively better growth resulted when levels of 1.0, 1.5, and 1.75 gm. pear were fed as a source of vitamin B (B_1), although none of these levels approximated normal growth. The food intake of the animals on the three levels of pear decreased per gram of rat as the supplements of pear increased, indicating that where the larger quantity of pear was consumed it replaced some of the basal ration (table 2).

Using the Sherman unit for expressing the vitamin B (B_1) content, the above results indicate that 0.73 gm. of dried Bosch pear (6.0 gm. of fresh pear) contains one unit of vitamin B. This estimate is based on gains in growth only and does not measure the polyneuritic or other symptoms of a deficiency. In view of our present limited knowledge of the various factors of the undifferentiated vitamin B, this quantity of pear may carry other unknown factors than vitamin B (B_1). Recently, Sherman ('31) has stated that—

in view of recent evidence that after several weeks on the basal diet, other vitamins, not yet well defined, may begin to be the limiting factors also, and thus complicate the measurement of Vitamin B, it now seems perhaps better to base the quantitative comparison upon the rate of gain during a period of four weeks beginning at the end of the depletion period.

Vitamin G (B₂) in Bosc pears

Progress in the study of vitamin G values in foods has been hampered by the fact it has been necessary to prepare extracts of a food rich in vitamin B and low in vitamin G, using a solvent which removes only a minimum amount of G. This involves much time and expense and it was practically prohibitive in this laboratory, nor was it possible to purchase vitamin B extracts free of vitamin G.

Through private communication with Dr. Hazel Munsell, of the Bureau of Home Economics, U. S. Department of Agriculture, information was obtained regarding the use of white corn in a diet as a source of vitamin B (B₁) but furnishing very little vitamin G. Later, Munsell published her results ('31), showing "that 30% of white corn in the Sherman and Spohn Vitamin B free diet does not supply an amount of vitamin G sufficient to promote growth or prevent the occurrence of symptoms of pellagra."

White corn was therefore used to the extent of 30 per cent of our basal diet which was identical with the Sherman and Spohn diet, with the exception of 2 per cent agar replacing 2 per cent of the starch. In comparing white corn with wheat as sources of B and G, Munsell found it to be slightly poorer than whole wheat in both vitamin B (B₁) and vitamin G. On a diet in which the vitamin G carried by 30 per cent white corn was the limiting factor, the growth of the young rats was shown to be proportional to the vitamin G of the test food, fed in addition to the basal diet. It is understood that in the assaying of foods for vitamin G values, all growth rates in the experimental period must be compared to that obtained on the basal diet containing a minimum quantity of vitamin G in the 30 per cent white corn.

Method. The variety and source of the white corn was the same throughout the experiment.³ Other constituents of the diet were prepared as for the assays of vitamin B complex and B. The experimental animals were from the same stock

³ Champ, White Pearl, Portland Seed Company, Portland, Oregon.

with the same breeding and nutritional history. The average weight of the animals at 28 days when put upon the experimental diets was 39 gm.

The depletion period ranged from 7 to 16 days. Five to eleven animals were used in each group. A few experimental animals were continued 50 per cent longer than the experimental period, to ascertain the effects of a longer period of vitamin G deficiency.

The animals were divided into nine groups as follows:

- Group I—Basal diet A (30 per cent corn).
- Group II—Basal diet B (B and G free).
- Group III—Basal diet A plus 0.5 gm. autoclaved yeast.
- Group IV—Basal diet A plus 0.5 gm. autoclaved yeast and 0.5 gm. dried pear.
- Group V—Basal diet B plus 0.5 gm. of dried yeast.
- Group VI—Diet A plus 0.5 gm. dried pear.
- Group VII—Diet A plus 0.75 gm. dried pear.
- Group VIII—Diet A plus 1.0 gm. dried pear.
- Group IX—Diet A plus 1.5 gm. dried pear.

Groups I and II constitute negative controls and were established because it was desired to secure symptoms of G avitaminosis, but primarily to test the vitamin G deficiency in the basal diet. Groups III, IV and V were planned as three positive controls and to determine whether basal diet A was adequate when vitamin G was supplied. Groups VI, VII, VIII, and IX were planned to test the various levels of pear fed as supplements to basal diet A as a source of vitamin G.

Results. The fact the average weekly gain was 1.92 gm. for group I (table 3) indicates that the constituents of the diet are not entirely free from vitamin G or that some other growth factor is present, or that the experimental animals were not depleted of the bodily reserves of a growth promoting vitamin. There are two possible sources for vitamin G in basal diet A, the casein and the white corn. Since the casein used was the same as that used in the purified diet (basal diet B) which produced a weekly loss in weight of 1.8 gm. (table 3, chart 2), death resulting, the presence of a very small amount of vitamin G must be assigned to the white corn as Munsell has reported ('31). The rats were considered depleted of their

store of vitamin G when the weight became stationary or there was a slight loss of weight. In group I growth was restricted to such a low level that when autoclaved yeast, a proved source of vitamin G, was added, the rate of growth increased 87 per cent.

TABLE 3
Effects of feeding Bosc pear as a source of vitamin G (B_2)

GROUPS	NUMBER OF RATS	AVERAGE INITIAL WEIGHT (GRAMS)	LENGTH OF DEPLETION PERIOD (DAYS)	AVERAGE WEIGHT AT END OF DEPLETION (GRAMS)	AVERAGE GAIN OR LOSS PER WEEK (GRAMS)	AVERAGE NET GAIN OR LOSS PER GRAM OF FOOD PER WEEK (GRAMS)
I. Basal diet A (vitamin B ₂) supplied in 30 per cent white corn)	7	41.0	12.0	49.4	+ 1.0	+ 0.06
II. Basal diet B (vitamin B complex-free)	5	47.0	- 1.8	- 0.07
III. Basal diet A + 0.5 gm. autoclaved yeast	11	42.0	12.0	49.0	+ 16.0	+ 0.24
IV. Basal diet A + 0.5 gm. autoclaved yeast + 0.5 gm. dried pear	8	41.6	14.0	53.4	+ 19.0	+ 0.30
V. Basal diet B + 0.5 gm. dried yeast	7	36.0	15.0	48.5	+ 15.1	+ 0.22
VI. Basal diet A + 0.5 gm. dried pear	8	39.9	11.0	48.9	+ 5.2	+ 0.25
VII. Basal diet A + 0.75 gm. of dried pear	7	35.4	11.0	45.3	+ 7.5	+ 0.17
VIII. Basal diet A + 1.0 gm. dried pear	6	41.0	11.0	49.7	+ 6.3	+ 0.17
IX. Effect of basal diet A + 1.5 gm. dried pear	6	38.0	9.0	44.0	+ 9.0	+ 0.20

Severe symptoms of G avitaminosis reported by numerous investigators were not obtained, although some characteristic symptoms were noted. During the 8 weeks' experimental period, the most easily discernible reactions, aside from re-

tardation in growth were sluggishness of movements and a lowering of vitality. Among the rats kept on the vitamin G deficient diet over a period of several months, the skin became dry and the fur lost its luster and fell out easily. Ears,

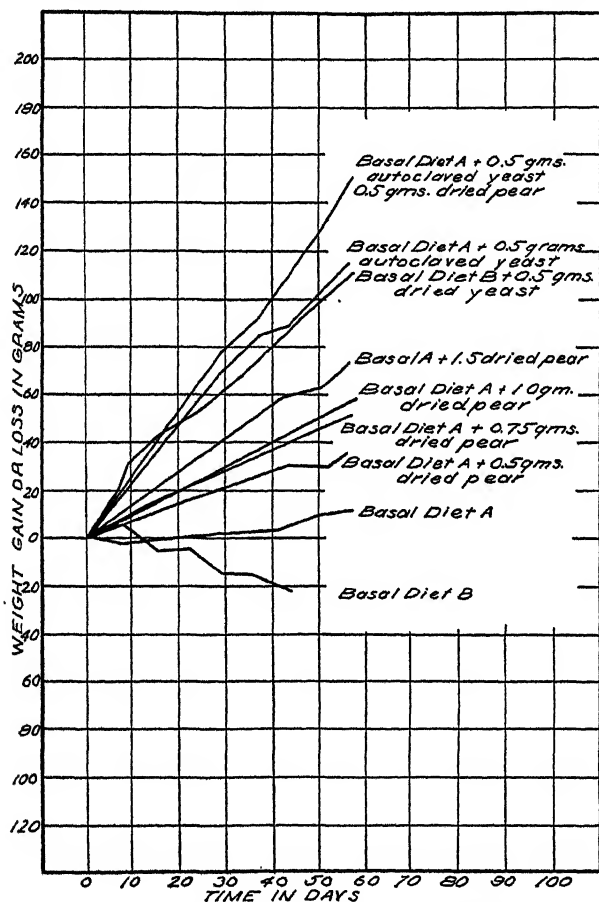


Chart 2 Results on growth of different amounts of dried Bosc pear as sources of vitamin G.

tail and feet assumed a bluish color. It has been shown that if the diet contains a little vitamin G, death is postponed and a dermatitis develops slowly (Sherman and Sandels, '31). Since our basal diet contained a minimal amount of vitamin G

in the white corn, this may account for the slow development of 'these' symptoms.

Group II furnishes evidence that the casein purification was satisfactory and that the animals' reserve store of vitamin G was soon exhausted if placed on a diet lacking this factor (table 3, chart 2).

Group III proved the efficiency of the autoclaved yeast to furnish sufficient vitamin G for normal growth when fed at a level of 0.5 gm. daily.

The addition of 0.5 gm. of dried pear to basal diet A plus 0.5 gm. autoclaved yeast (group IV) indicates that the pear was supplying some limiting growth factor when the weight gains for the 8-week period are compared, but if the comparison be confined to a 5-week period the average gain per week of these two groups was practically identical.

Sherman ('31) tentatively suggests that all quantitative determinations be based on a 4- or 5-week experimental period, since the rate in gain or loss is maintained fairly uniform during the first 4 weeks, and usually between the fifth and seventh week of the experimental period the growth curve tends to flatten. Therefore, interpretation of data should consider the possibility of a limiting factor appearing during the latter part of the experimental period of 8 weeks.

Group V was introduced as a check against the other positive controls, groups III and IV. The respective gains per week in these three groups were 15, 16 and 19 gm. (table 3). It is evident that the purified diet B supplemented with yeast supplied adequate amounts of vitamin B and G for normal growth.

Groups VI, VII, VIII, and IX show the effects of the addition of 0.5, 0.75, 1.0, 1.5 gm. of pear, respectively, as a source of G to basal diet A (table 3, chart 2). The gains in weight of animals in these groups were in proportion to the amounts of pear fed. It may be assumed, therefore, that the dried pear was supplying vitamin G. Translating these data into Sherman-Bourquin units, it appears that 0.59 gm. of dried pear (4.64 gm. of fresh pear) contain a unit of vitamin G

and also comparing Bosch pear as a source of vitamin G with autoclaved yeast, it would require 2.97 gm. of dried pear (24.87 gm. of fresh pear) fed daily, to produce the same rate of growth as 0.5 gm. of autoclaved yeast. In other words, autoclaved yeast is approximately sixty times as good a source of vitamin G as dried Bosch pear.

CONCLUSIONS AND SUMMARY

1. The vitamin B complex, vitamin B and vitamin G, of Bosch pears has been studied and tentative units for each are suggested.

2. Expressed as the quantity of dried pear yielding one Sherman unit, 1.21 gm. (10.03 gm. fresh pear) will yield 1 unit of vitamin B complex. Translating this in terms of units per ounce, 1 ounce of fresh pear furnishes 2.8 units of vitamin B complex.

3. As a source of vitamin B, 0.73 gm. dried Bosch pear (6.0 gm. fresh pear) furnishes 1 unit of vitamin B, or 1 ounce fresh pear furnishes 4.7 units of vitamin B.

4. As a source of vitamin G, 0.59 gm. of dried pears (4.64 gm. of fresh pear) contains 1 unit of vitamin G, or 1 ounce of fresh pear furnishes 6.1 units of vitamin G.

5. It appears, therefore, that Bosch pears are a somewhat richer source of vitamin G than vitamin B.

6. Results show that white corn, when fed as 30 per cent of basal diet B, supplied enough vitamin B to prevent symptoms of polyneuritis and vitamin B deficiency, but does not supply enough vitamin G to promote more than very slight growth (1.9 gm. weekly).

7. Autoclaved yeast at pH 5 when fed at a 0.5 gm. level, supplemented the 30 per cent white corn in vitamin G and normal growth was obtained on this level.

8. When vitamin B is adequately supplied by white corn, vitamin G is furnished by Bosch pear in the same ratio as the quantity of pear supplied in the diet.

9. In laboratories not equipped to prepare extracts of vitamin B for use in the assaying of foods for vitamin G, fairly satisfactory results may be obtained by use of the Munsell method in which white corn furnishes the vitamin B.

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FAT SOLUBLE VITAMINS

XXXV. THE OPHTHALMOGENIC PROPERTIES OF CERTAIN RATIONS LOW IN VITAMIN A ¹

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Ophthalmia, as a characteristic symptom of vitamin A deficiency, is subject to variations in its occurrence, as is evident from the reports of workers in different laboratories. Osborne and Mendel ('21) report an incidence of ophthalmia in 50 per cent of their animals; Stephenson and Clark ('20), 65 per cent; Emmett ('20), 98 per cent, and Sherman and Munsell ('25), 95 per cent. In our experience during the last 3 years, during which we fed approximately 500 rats on a ration uniformly free from vitamin A, we observed an incidence of ophthalmia in 70 per cent of all animals at the end of 5 weeks. This figure was increased to 95 per cent at the end of 6 weeks. As a matter of fact, many papers dealing with biological assays for vitamin A make no mention of the incidence of ophthalmia, presumably because such symptoms were either very mild or entirely absent. Of course, some of the variations in the experience of different laboratories may have been due to difference in the storage reserves of the animals, which may have been sufficient to delay the onset of all symptoms of deficiency beyond the period of observation; but this does not explain why symptoms of ophthalmia should have failed to appear before death from vitamin A deficiency ultimately resulted. It is possible that

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the strains of rats used in some laboratories may have had a predisposition to ophthalmia, and that other strains, in turn, may have possessed a primary weakness in certain organs leading to an early failure of function and thereby death. We, for instance, were led to suspect such a situation when at one time in a number of litters the majority of the animals died precipitously from hemorrhages in the pancreas without any other indications of lack of vitamin A. This experience, however, has not been duplicated within the last year or more.

The high incidence of ophthalmia in our work in contrast with the observations made in other laboratories led us to study the specific role of various dietary factors which might be responsible. In these studies we varied the composition of our basal starch ration by substituting for the starch various grains and their by-products and by putting in yeast, agar, various salts, and additional protein. The protein was added not only to determine whether it had any direct effect, but also to determine the effect of better growth which it made possible. The yeast was put in for the same reason, and the salts were modified to determine if by chance the action destructive of vitamin A, well known to be exerted by iron salts, for instance, would reveal the presence of vitamin A in the basal diet. In some of these rations we incorporated the cereal ingredients after they had been cooked at a high temperature, such as is used in the preparation of our cooked starch, which has been a common ingredient in our usual basic vitamin A-free diet. In other cases we moistened the ration with water or mixed it with oil to make it less dusty, and thereby possibly less irritating in an incipient ophthalmic condition.

We shall not present all of our results, inasmuch as many of them have no bearing on the ultimate outcome. But, in general, we can state that as a result of these experiments we have come to the conclusion that other factors besides the mere freedom of the ration from vitamin A may be responsible not only for an acceleration in the rate at which ophthalmia

develops, but also for the severity which it attains in the course of the first few weeks. Although our experiments were not designed to reveal whether or not these factors would have determined the absolute occurrence or non-occurrence of ophthalmia if the experiment had been sufficiently prolonged, yet we are of the opinion that in some cases death ultimately might have resulted entirely from causes other than ophthalmia.

EXPERIMENTAL

Our experiments were carried out with young rats weighing 40 to 50 gm. at an age of 22 to 27 days. They were so distributed among the different lots of each series that litter representatives were present in each lot. Each lot was composed of five animals. The animals were weighed weekly until deficiency symptoms appeared—after that they were weighed at more frequent intervals. The rations were fed in tin feeding cups about $2\frac{1}{2}$ inches high and $3\frac{1}{2}$ inches in diameter. Vitamin D was furnished in all rations by the addition of 0.1 per cent of yeast which had been irradiated so as to contain one rat unit per milligram (Steenbock, et al., '32). In some cases records of the food consumed by each lot were kept for short periods. The animals were carefully examined for ophthalmia every few days after the first 3 weeks. For purposes of comparison the ophthalmias were arbitrarily classified as given in the footnote to table 2.

After the symptoms of vitamin A deficiency had become pronounced, carotene was administered in some cases for the purpose of determining the curative and growth promoting properties of the ration when vitamin A activity was present. We entertained the hope that these data would enable us to select a ration in which certain variables would have been omitted and the ration thereby made more suitable for vitamin A assays. Our experiments will be discussed in series which were run consecutively as the problem developed.

Series 1. Comparison of different cereal grains. This series actually consisted of two series. They were run con-

secutively to verify the original observations, but the results are presented collectively to save space. In these experiments we made a comparison between rolled oats, white corn, wheat, polished rice, and cornstarch for their ophthalmogenic and growth promoting properties. Ration F (table 1), containing the cornstarch, represented our most commonly employed ration which we have frequently designated as our basal synthetic ration for vitamin A work. In this the starch had been cooked by autoclaving with water at a temperature of 117° for 2 hours. It had then been dried thoroughly at 60° for 1 week. The casein was a commercial product in which

TABLE 1
Composition of rations¹

		CASEIN	NaCl	CaCO ₃	SALTS 40	YEAST	AGAR
(a) Rolled oats	86	12	1	1
(b) Rolled oats	80	18	1	1
(c) White corn	86	12	1	1
(d) Wheat	86	12	1	1
(e) Polished rice	80	12	1	1	..	6	..
(f) Cornstarch (cooked)	69	18	4	7	2

¹ All rations contained 0.1 per cent of dried yeast obtained from standard brands and irradiated in our laboratory so as to contain one unit (Steenbock) of vitamin D per milligram.

all vitamin A had been destroyed by heating it in a finely ground condition in thin layers in shallow pans for 2 weeks at 90°C. The composition of the salt mixture has already been described (Steenbock and Nelson, '23). The yeast² had been previously tested for vitamin B potency in our laboratory.

In table 2 there are presented the results on the production of ophthalmia and the changes in weight of all animals after they had been on experiment from 26 to 35 days. As seen from the table, in series 1 there occurred pronounced growth of animals on the rice ration, together with an early incidence of ophthalmia. In this respect the cornstarch ration was a

² Obtained from Northwestern Yeast Co., Chicago

LOT NOS.		RATIONS		OPHTHALMIC TOTALS ¹										WEIGHTS (GRAMS)	
Series 1. Comparison of grains															
		(28)	(33)	(35)	(38)	(40)	(42)	(33)	(35)	(37)	(38)	(40) ²			
161, 167	Roller oats (12 per cent casein)	0	0	9	11	11		84	85	84	84	84			
162, 168	Roller oats (18 per cent casein)	0	0	5	8	8		82	84	83	83	84			
163, 168	White corn	0	0	2	3	3		86	87	86	85	85			
164, 170	Wheat	0	0	0	0	1		108	112	113	112	113			
165, 171	Polished rice	7	14	15		121	118	110			
166, 172	Cornstarch	0	7	11		106	109	107			
Series 2. Effect of 6 per cent yeast															
		(33)	(35)	(37)	(38)	(40)	(42)	(33)	(35)	(37)	(38)	(42)			
190	Roller oats (12 per cent casein)	4	4	6	6	7	8	97	98	99	100	102			
191	Roller oats (18 per cent casein)	0	2	6	6	7	7	124	128	133	131	131			
192	White corn	2	5	8	8	122	119	128	116	...			
193	Polished rice	12	12	15	15	103	100	97	96	...			
194	Cornstarch	2	2	4	8	9	..	108	109	109	112	105			
195	Cornstarch (9 per cent casein)	0	0	4	12	78	...	80			
Series 3. Rice from different sources															
		(26)	(28)	(30)	(35)			(26)	(28)	(30)	(35)				
200	Cornstarch	0	0	4	13			108	108	107	108				
201	Polished rice no. 1	3	7	14	..			104	104	98	88				
202	Polished rice no. 2	4	5	10	13			124	126	123	107				
203	Polished rice no. 3	8	12	14	..			119	123	114	111				
204	Polished rice no. 4	3	4	8	..			124	118	102	...				
205	Polished rice no. 5	4	11	14	..			119	120	104	96				
Series 4 and 5. Effect of heat, water, or fat															
		(28)	(30)	(32)	(35)			(28)	(30)	(32)	(35)				
234	Rice starch cooked	0	0	0	2			83	85	86	89				
236	Cornstarch	0	1	2	6			118	119	122	122				
237	Polished rice	8	9	11	..			132	130	130	...				
238	Polished rice (fed moistened)	0	0	1	2			117	...	124	...				
241	Polished rice (cooked)	0	0	0	0			58	...	55	53				
242	Polished rice (plus oil)	0	0	4	8			129	...	132	125				
243	Cornstarch (plus oil)	0	0	2	7			127	...	131	133				

¹ Ophthalmic totals were obtained by multiplying the degree of ophthalmia by the number of animals afflicted in each lot. To make all the data comparable the values for series 1 were divided by 2, because there were ten animals in each group instead of five. The degrees of ophthalmia were differentiated arbitrarily as follows: First degree, bareness of lids with or without exudate; second degree, bareness of lids with slight swelling or slight bleeding; third degree, marked swelling of lids with or without hemorrhage and with or without necrosis of conjunctiva; fourth degree, purulence of the eyeball together with any or all of the aforementioned symptoms.

² The figures in parentheses represent the number of days that the animals had been on experiment.

close second. Wheat also gave good growth, but the incidence of ophthalmia was much delayed. This was in harmony with earlier results from this and other laboratories which have shown that the wheat kernel contains estimable quantities of vitamin A (Steenbock and Coward, '27; McCollum and Davis, '15). Rolled oats and white corn both allowed but little growth, and both produced ophthalmia somewhat slower than the cornstarch ration, although much earlier and more severely than the wheat. That the rice and cornstarch rations were suitably constituted to reveal differences in vitamin A content was revealed by the fact that both of them gave a favorable response to the addition of carotene. When 5 gamma carotene were given daily to each animal, the rice-fed animals gained 30 gm. in 4 weeks and the starch-fed animals 35 gm. With the corn ration the addition of carotene also gave good growth, but with the oat rations the results were mediocre. Growth on the ration containing 18 per cent casein was somewhat better than with 12 per cent casein.

Series 2. The effect of yeast additions. Inasmuch as in series 1 the combined results of earliest production of ophthalmia and the best growth had been obtained with the two rations, namely, rice and cornstarch, which also contained 6 to 7 per cent yeast, that series of experiments was repeated with the addition of yeast to the other rations as well. However, from this series the wheat ration was omitted as it obviously contained too much vitamin A to make its inclusion worth while. In these experiments the addition of yeast still left the polished rice ration outstanding for its early production of ophthalmia. Although growth was increased in the other rations, except where the protein level had been too low, it was made evident that the early appearance of ophthalmia was not directly related to the rapidity of growth.

Series 3. The effect of rice from different sources. To determine if the early production of ophthalmia is a property common to rice in general, we secured rice from five different

sources and fed it as usual in ration E (table 1). Ration F, containing cornstarch, was again used as a control. As shown in table 2, there occurred some variations in the incidence of ophthalmia and also in the production of growth; but we are rather doubtful as to their significance in view of the small number of animals employed. It can, however, be stated that all the rice rations were without question more ophthalmogenic than the control ration.

Series 4 and 5. The effect of various treatments of rice and various supplements. These series were run to determine if the physical state of the polished rice ration was responsible for its ophthalmogenic properties. To this end polished rice was fed in one ration in an untreated form, except for its being ground to an impalpable flour. For comparison therewith there were fed identical rations, except that in one case they contained cooked rice starch, in another case ground rice moistened with water, in a third case ground cooked rice, and in a fourth case ground rice mixed with 5 per cent cottonseed oil. For control purposes there was fed the usual cornstarch ration F with and without 5 per cent cottonseed oil.

A comparison of the degree of ophthalmia obtained from feeding the different rations showed rather striking results. As usual, ration E, containing the untreated ground rice, produced ophthalmia most quickly. When ration E was moistened and when it was treated with oil it produced ophthalmia at the same rate as the cornstarch ration. In other words, the addition of oil did not affect the incidence of ophthalmia on the starch ration, but it did retard it with the rice ration.

The growth results on these rations showed considerable variations. The best growth was obtained with the untreated rice ration or the untreated rice ration or cornstarch ration plus oil. The animals averaged 130 gm. in weight after 4 weeks. On the cornstarch ration alone, or on the raw rice ration fed moist, the animals averaged from 115 to 120 gm. in 4 weeks. On cooked rice practically no growth was obtained, the average weight after 4 weeks being 58 gm.

The inferences which might be drawn from the above findings are that the physical state of the rice was an important factor in determining its ability to produce ophthalmia. In such instances where the rice particles were made less irritating as a result of disintegration by cooking or by being weighted down with water or oil, ophthalmia was delayed in its appearance. It appeared very probable that the early incidence of ophthalmia on the rice ration was due to an irritating effect exerted by fine particles of starch which entered the eyes. As the evidence for this was entirely circumstantial, we decided to obtain further data on the exhaustion of vitamin A reserves by the vaginal smear test of Evans and Bishop ('22). This test is based on the fact that when the vitamin A stores of an animal become depleted cornified cells become persistent in the vagina. These can readily be followed in their occurrence by microscopic examinations of smears made daily.

Series 6. Vaginal smear experiments. Twenty-four rats weighing 50 to 60 gm. were divided into two groups. One group was given the cornstarch ration and the other the untreated polished rice ration. Vaginal smears were made as soon as the vaginal orifice made its appearance. This practice was continued for 2 weeks after cornified cells had persisted.

On the cornstarch ration of the twelve animals on experiment, nine showed their first smears characteristic of vitamin A deficiency in the period from the twenty-fifth to the thirty-eighth day. The average for the group was 31 days. Three animals remained immature. On the rice ration the first characteristic smears appeared from the twenty-fifth to thirty-sixth day, with an average of 30 days. One animal remained immature. These data show a remarkable uniformity in the appearance of the characteristic smears. However, on the rice ration ophthalmia again appeared earlier, and after 6 weeks the ophthalmic total, expressed according to the system used in table 2, was twenty-one for the rice ration and eight for the cornstarch ration. These results therefore indicate that while the vitamin A stores of the

animals on the two rations were depleted at the same rate, the rice ration without question possessed greater ophthalmogenic properties.

We also made the observation that our rice fed animals were more subject to diarrhea than the animals on cornstarch. On the cornstarch rations, diarrhea, as a matter of fact, was rarely observed and then only when the avitaminosis was far advanced. It might be proposed that the diarrhea was due to the coarseness of the rice particles and its high specific gravity, which made it more irritating. In reply to this it should be stated that while the specific gravity of the untreated rice ration was high, a rice flour ration was much lower; in fact, lower than the cornstarch ration, and yet both produced ophthalmia to an equal degree.

The relative coarseness of the cereal ingredients was not a factor, because the cereal products were all ground to a powder in a ball mill and microscopic examination failed to reveal any difference in shape of particles or sharpness of edges. Yet it was noteworthy that untreated rice was apparently digested with some difficulty because starch grains were frequently found in the feces.

SUMMARY

It appears as a result of our experiments that the physical qualities of a ration which may allow irritating ingredients to lodge in the eyes of an experimental animal are factors which should be given consideration in the production and possible cure of ophthalmia. As the incidence of ophthalmia frequently leads to a secondary effect on growth, the physical state of the ration may have an effect on growth as well. It is accordingly recommended that when rations low in vitamin A are to be used for assay purposes, they should be so treated by cooking, moistening, or otherwise, as to allay their dust producing properties. In addition it follows from our observations on growth and the production and cure of ophthalmia that a basal ration consisting of cooked polished rice 80, heated casein 12, sodium chloride 1, calcium carbonate 1, and

yeast, high in vitamin B, 6, constitutes an excellent basal ration for determining vitamin A in concentrates such as cod liver oil and the like. This ration, furthermore, has the advantage of low cost as its salt ingredients are especially inexpensive.

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THE EFFECT OF ACID ASH AND ALKALINE ASH FOODSTUFFS ON THE ACID-BASE EQUILIBRIUM OF MAN

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INTRODUCTION

While the evidence (Bischoff, '32) to date indicates that sustained changes in CO_2 content and plasma pH of humans outside the so-called normal limits cannot be produced by the ingestion of foodstuffs, the possibility and significance of changes within this limit have not been eliminated. Cullen and Earle ('29) have recently studied the normal changes in pH and CO_2 content of the blood during the course of the day. The normal shift in pH may be 0.07 more alkaline. The normal limits for venous blood plasma, according to these authors, is pH 7.33 to 7.51, and CO_2 content, 55 to 74 volumes per cent. Haldane ('21) showed that by taking 15 to 20 gm. of ammonium chloride per day an acidosis could be produced. He was able to lower the bicarbonate of his blood by one-half. This amount of ammonium chloride will reduce the pH of the blood by 0.2. The daily administration of 45 gm. of sodium bicarbonate was found necessary to change the reaction of the blood to a similar extent in the opposite direction. Smaller amounts of these substances upon administration will produce transitory effects (Palmer and Van Slyke, '17). It would require 18 pounds of oranges to have an alkaline ash content equivalent to 40 gm. of NaHCO_3 , and 4.5 pounds of lean beef,

or 2 pounds of oysters, to have an acid ash content equivalent to 15 gm. of NH_4Cl .

Michalowsky ('30) has shown that the ingestion of 2.5 gm. of phosphoric acid daily, for a period of 6 weeks, had no effect upon the alkali reserve. The equivalent of lean beef in acid ash content is approximately a pound, so that Michalowsky's work does not rule out the possibility of producing a change in the blood by diet. Michalowsky also studied the effect of a mixed diet of meat, fat, and wheat on the alkali reserve. He observed no change. The exact composition of his diet is not available, nor the urine acidity. The foregoing facts raise the question as to the minimum amount of acid or alkali required to change the pH of the blood, it being pertinent to decide whether these amounts could be liberated in the consumption of a foodstuff.

The study of the effect of the ingestion of acid ash and alkaline ash foodstuffs on temporary shifts of the acid-base equilibrium of the body is complicated by the alkaline tide. In general, the acid and alkaline ash of the foodstuffs administered in this study have not been considered, an exception being the work of Fiske ('21) who found that the maximum alkalinity of the urine, after a protein meal, is often reached at a time when the excretion of phosphate and sulfate is at a maximum. Since fasting normal subjects show a diuresis with an alkaline tide in the morning (McCorvie, '25) and a fall in CO_2 combining power which may reach 5 volumes per cent, some doubt must be cast on the significance of experimental results which have apparently shown a relation between ingestion of foodstuff and change in acid-base equilibrium. In reviewing the older literature, one wonders whether the effects of exercise on the acid-base equilibrium were sufficiently controlled.

PLAN

The present study was concerned with two major problems: to determine whether it was possible to produce 1) a sustained shift or 2) a temporary shift in the acid-base equilibrium of

the blood following the ingestion of acid ash or alkaline ash foodstuffs.

The minimum amount of sodium citrate which would shift the acid-base equilibrium of the morning fasting blood significantly was determined and administered for 40 days. Sodium citrate was chosen because it is a natural alkaline ash-producing constituent of foods and introduces the factor of citric acid oxidation.

In another experiment, an individual was placed on his usual diet and then on a diet containing acid ash foodstuffs, and finally on a diet containing alkaline ash foodstuffs.

In a third experiment, the same individual was placed on an acid ash diet containing the acid ash equivalent of 3 pounds of beef. This diet was necessarily high in protein.

In studying temporary effects, that is, changes that might be induced during the course of 8 hours following the ingestion of a food, a pound of steak, a quart of milk, a pound of bananas or a quart of orange juice was eaten.

In all cases, except those in which the subject was required to maintain normal activity, blood samples were taken after a half hour of bed rest. In studying temporary changes, the subject was at bed rest during the entire course of the experiment, unless otherwise indicated. CO_2 , oxygen content, and oxygen capacity were determined for whole blood. Plasma pH and CO_2 were also determined on plasma as drawn. For the corresponding intervals the urinary determination of titratable acidity, ammonia, and pH was made.

Since the primary interest was in plasma pH and alkali reserve, the data have been presented to show these values. The plasma bicarbonate is the best criterion of alkali reserve. In order that the comparison of all plasma bicarbonate values might be strictly comparable, the values presented were recalculated for completely oxygenated blood at the pH drawn, using the well-known constants and relations of Van Slyke ('31). In most instances, plasma CO_2 content was also determined, since this value serves as an excellent check for the whole blood data.

The analytical methods used were: whole blood CO_2 , oxygen capacity and oxygen content according to Van Slyke and Neill ('24) and Van Slyke and Sendroy ('27), titratable acidity and ammonia according to Folin ('03) and Folin and Bell ('17), phosphorus according to Youngsberg ('30), sulfur according to Folin ('05). Plasma pH was determined by Cullen's method as modified by Bischoff, Long, and Hill ('30).

RESULTS

Normal variation. From four to seven pre-breakfast blood determinations were made at intervals for each of the six individuals studied. The greatest deviation between any two plasma bicarbonate values for any one individual was 3 m.Mol. The individual extreme values were: 26.9 to 29.2, 28.0 to 28.7, 28.5 to 30.0, 27.2 to 29.0, 24.0 to 27.0, 25.8 to 26.5. It would, therefore, be doubtful whether a 3-m.Mol. change in an isolated experiment, in which data were collected before and after the ingestion of a foodstuff, should be considered significant, as has been repeatedly done in the past. In the present studies, no change is considered significant if the values fall within the range established by a series of fasting values. Consecutive fasting values for a 3-hour interval were determined in two instances. Changes of a millimol were noted.

The normal variation of the individual's pre-breakfast pH covered 0 to 0.04 pH (three to five determinations on each individual). The impression gathered in earlier studies of Bischoff, Long, and Hill ('30, '31) and Bischoff, et al., ('30) that each individual has a characteristic pH range is extended. It should be noted that, after an interval of 2 years, the values of subject L.C.M. shifted from the lower normal range to the upper range. The values for subject Mc. are the highest normal values so far found.

Effect of sodium citrate. The subject was hospitalized during the period of experimentation. The bleeding was done in the morning before breakfast. The first blood sample was collected for the determination of control values. The subject was then given 8 gm. of citrate per day for 7 days, when

blood data were collected. Following this period, citrate was given daily in 4-gm. doses, a blood sample being collected after 9 days. Thirty gm. of sodium citrate were then given daily for 40 days. Three blood samples were collected during this period, the first, 4 days after the initial dosing. Following this period, the basal diet was resumed for 10 days.

The low doses of citrate had no effect upon either the total CO_2 or blood pH. There was a remarkably close agreement for total plasma bicarbonate, the maximum variation being less than 1 m.Mol. per L. All the pH values but one were the same. The exceptional value was 0.05 pH more acid.

TABLE 1

Effect of sodium citrate administration upon the acid-base equilibrium of pre-breakfast blood

CITRATE ADMINISTRATION	PERIOD	URINE pH	BLOOD PLASMA ¹	
			BHCO ₂	pH
			m.Mol. per L.	
Control	Initial	7.2	25.8	7.45
8 gm. citrate daily	7 days	7.8	25.6	7.40
Control	Initial	7.6	26.0	7.45
4 gm. citrate daily	9 days	8.0	25.8	7.45
30 gm. citrate daily	4 days	8.6	28.2	7.48
30 gm. citrate daily	22 days	8.4	27.9	7.48
30 gm. citrate daily	39 days	8.4	28.2	7.45
Control	10 days	6.8	26.5	7.45

¹ Recalculated for completely oxygenated blood at pH drawn.

These values were all within the normal established pH range. During the final period of sodium citrate administration, the plasma CO_2 rose 3 m.Mol. per L. The plasma pH rose 0.03 in two cases, with no change in a third. All these values are again within the normal range, but they indicate a shift to the alkaline side. One determination was made after breakfast and after the ingestion of 5 gm. of sodium citrate. The blood pH was elevated 0.03, the plasma bicarbonate 3 m.Mol. per L. over the basal values. These values are also in the normal range. The results are given in table 1.

Acid diet. The subject was asked to eat the diet he regularly followed for a period of 6 days. Twenty-four-hour

urine specimens were collected daily, the urine pH and titratable acidity being determined. Acid-base data were collected from blood drawn on two mornings before breakfast. The subject was then placed for 9 days on an alkaline ash diet. This diet consisted of a mixture of neutral and alkaline ash foods. Acid-base data of the blood were determined three times. An acid ash diet was then followed for 9 days. This diet consisted of a mixture of neutral and acid ash foods. Acid-base data for the blood were determined twice.

TABLE 2

Effect of ash variation in diet upon the pre-breakfast acid-base equilibrium of the blood

DIET	PERIOD	24 HOUR URINE		BLOOD FINDINGS (PLASMA) ¹	
		pH	Titratable acidity cc. N/10	pH	BHCO ₃ m.Mol. per L.
Usual	6 days	5.8 ± 0.15	234 ± 27		
Usual	4th day	6.2	295	7.40	29.0
Usual	5th day	5.8	200	7.41	26.9
Basic	9 days	6.9 ± 0.15	130 ± 31		
Basic	2nd day	6.4	68	7.45	27.2
Basic	4th day	7.2	98	7.41	28.8
Basic	9th day	7.2	113	7.44	29.2
Acid	9 days	5.9 ± 0.12	262 ± 17		
Acid	4th day	5.8	273	7.40	28.5
Acid	9th day	6.4	246	7.44	29.2

¹ Complete oxygenation.

Probably the most interesting result of this experiment was that the urine pH during the uncontrolled diet period was as acid as that in the period when a carefully selected acid diet was consumed. The subject responded classically to the change in alkaline diet, the urine pH shifting from 5.8 to 6.9. No change in plasma pH or plasma CO₂ outside the normal expected variation was observed. The total CO₂ of the plasma was in the upper limits of normal, showing a maximum variation of 2.3 m.Mol. per L., the highest value being observed on the acid diet, the lowest on the control diet. The plasma pH fluctuated in the region of the lower limits of normal (table 2).

Since this experiment showed rather conclusively that fairly acid ash and alkaline ash diets had no effect upon the acid-base equilibrium of the blood, a more drastic experiment was planned. A period of 2 years elapsed between the first and second experiments. The same subject was studied. The diet consisted almost entirely of meat (exception 20 gm. of rice) with an acid ash equivalent of 3 pounds of lean beef. Brains, ham, chicken, veal, beef, and eggs were used. After 2½ days of this diet régime, the subject became nauseated, when he was returned to a more rational diet. It was regretted that a longer period on the high protein-acid ash diet could not be followed. If the response to ammonium chloride ingestion may be used as a criterion, an acid-base equilibrium would have been reached. The results showed no significant change in blood pH after 2 days on the diet. The 1 m.Mol. drop in plasma CO₂ might be significant, as it was the lowest value observed for the individual studied. Of great interest was the increase in total CO₂ the morning following the cessation of the acid diet. At this time a diarrhea developed. We have previously pointed out that Page ('23) observed an increase in alkali reserve in rabbits which developed a diarrhea. At this period the pH was still normal.

While the acid-base data of the blood again showed that the acid ash produced no decided shift, the other data which were gathered bear inspection. There was a lag in nitrogen excretion for a day and a half after the high protein diet was stopped (table 3). The blood urea nitrogen rose considerably. Ammonia excretion increased appreciably. No albumin or casts were found in the urine. These are all well-known observations.

Steak (temporary effect). Data for four individuals, including six experiments, were collected to determine the effect of the ingestion of 1 pound of steak (equivalent roughly to 50 cc. of normal acid). For subject Mc., a fall of 3 m.Mol. below the control value was noted at the eighth hour on two occasions, one under complete rest, the other under normal activity. However, 8 hours after the ingestion of a quart of

TABLE 3
Effect of ingestion of approximately 200 cc. normal acid as acid ash food

DATE	PERIOD	URINE FINDINGS				BLOOD FINDINGS				DIET			
		S.G.	pH	Alb. or casts	Total N-24-hr.	NH ₄ 24-hr.	Plasma BHO ₂ ¹	Plasma pH	Urea N	Acid ash	Carbo-hydrate	protein	Fat
					Gm.	Gm.	m.Mol./L.		Mg./100 cc.	Gc. N			
May 11	Control	1.034	5.5	...	10.0	0.7	26.5	7.50		202	125	243	178
May 12	Exp. diet	1.030	5.8	...	21.6	0.7				190	116	204	112
May 13	Exp. diet	1.030	5.3	...	17.5	0.7				189	142	93	127
May 14	Exp. diet	1.020	5.2	...	20.1	1.0	25.2	7.45	34.0				
May 15	Recovery	1.023	5.8	...	13.7	0.5	29.0	7.47	22.5				
May 16	Recovery	1.016	6.0	...	8.9	0.3							

¹ Recalculated for completely oxygenated blood at pH drawn.

milk (an essentially neutral ash food) a fall of 2 m.Mol. was noted (table 4). The maximum effect that could be attributed to the acid ash of the steak was therefore a decrease of 1 m.Mol. For subject B., a fall of 1 m.Mol. was noted the fifth hour, with a return to the control value the eighth hour. In this experiment subject B. was under normal activity. The experiment showed no change when subject B. was under rest. In the case of subjects H. and C., the decrease by 1 m.Mol. has no significance, since a similar fall was noted under fasting, following orange juice, or following 50 cc. of 7 per cent alcohol.

Orange juice and bananas. The four experiments concerned with orange juice or bananas showed no effect on the acid-base equilibrium. The small variation in values was well within the expected normal variation and was doubly controlled by taking fasting values at the same time of the day (table 4).

Urinary changes. Changes in the urinary constituents were determined during the periods of diet experimentation so that blood samples might be collected at the time when kidney excretion indicated maximum acid-base changes. In four out of the five experiments concerned with steak, the rate of phosphorus excretion was descending at the period of maximum urine acidity (table 5). It seems important to emphasize that there is no correlation between either urinary pH or titratable acidity and plasma bicarbonate. In the case of subject B. (five determinations), the plasma bicarbonate fluctuated between 27 and 28 m.Mol. at the time the urinary acidity was in the range 7.0 to 7.5; at the pH range 4.9 to 5.5, the bicarbonate fluctuated between 27 and 29 m.Mol (five determinations, table 4). Similar results were obtained for the other subjects studied and it seems unnecessary to present the correlation chart. In the case of subject B., the plasma bicarbonate values were the same when the rates of urinary excretion of titratable acidity and ammonia were markedly different (986 against 579 cc. 0.1 N acid per 24 hours). Rates of 106 and 230 showed identical plasma bicarbonate values

TABLE 4

Temporary acid-base plasma changes following ingestion of various foodstuffs

SUBJECT	PERIOD	CONDITIONS	PLASMA	
			BHCO ₃ ¹	pH
			<i>m.Mol. per L.</i>	
H.	8:30 a.m. Feb. 8	Fasting	28.0	7.46
	11:30 a.m. Feb. 8	Fasting	26.7	7.47
	8:50 a.m. Feb. 12	Fasting	28.5	7.48
	11:50 a.m. Feb. 12	3 hours after 1 quart of orange juice	26.5	7.49
	7:30 a.m. Feb. 18	Fasting	28.2	7.49
	1:30 p.m. Feb. 18	6 hours after 1 pound of steak	27.0	7.51
	9:00 a.m. Feb. 24	Fasting	28.7	7.47
	10:00 a.m. Feb. 24	1 hour after 50 cc. of 7 per cent alcohol	27.2	7.45
C.	9:00 a.m. Feb. 10	Fasting	26.1	7.49
	12:00 a.m. Feb. 10	Fasting	27.1	7.47
	9:00 a.m. Feb. 18	Fasting	24.0	7.49
	12:00 a.m. Feb. 18	3 hours after 1 quart of orange juice	26.0	7.50
	9:00 a.m. Feb. 25	Fasting	25.5	7.50
	4:30 p.m. Feb. 25	7.5 hours after 1 pound of steak	24.1	7.48
	9:00 a.m. Mar. 3	Fasting	26.0	7.47
	9:30 a.m. Mar. 3	0.5 hour after 50 cc. of 7 per cent alcohol	24.2	7.50
B.	9:00 a.m. Sept. 1	Fasting	26.3	7.51
	4:30 p.m. Sept. 1	7.5 hours after 1 pound of bananas	26.0	7.50
	9:30 a.m. Feb. 19	Fasting	28.0	
	10:30 a.m. Feb. 19	After 1 pound of steak	27.0	
	12:30 p.m. Feb. 19	After 1 pound of steak	28.8	
	2:30 p.m. Feb. 19	After 1 pound of steak	28.0	
	8:30 a.m. Aug. 26	Fasting	27.8	
	11:05 a.m. Aug. 26	After 1 pound of bananas	27.8	
	2:20 p.m. Aug. 26	After 1 pound of bananas	27.0	
	3:15 p.m. Aug. 26	After 1 pound of bananas	28.2	
	8:45 a.m. Aug. 31	Fasting	27.2	
	2:00 p.m. Aug. 31	After 1 pound of steak	26.0	
	4:45 p.m. Aug. 31	After 1 pound of steak	27.2	
	8:30 a.m. Sept. 14	Fasting	29.0	
	2:30 p.m. Sept. 14	After 1 quart of milk—normal activity	28.0	
M.	7:00 a.m. Mar. 25	Fasting	30.0	7.50
	7:00 a.m. Mar. 26 ²	Fasting	29.2	7.53
	3:00 p.m. Mar. 26	After 1 pound of steak	26.2	7.49
	7:00 a.m. April 1	Fasting	29.5	7.52
	7:00 a.m. Aug. 19	Fasting	27.5	7.48
	11:00 a.m. Aug. 19	After 1 pound of steak	28.0	7.48
	3:00 p.m. Aug. 19	After 1 pound of steak	24.5	7.48
	7:00 a.m. Aug. 22	Fasting	29.5	7.49
	3:00 p.m. Aug. 22	After 1 quart of milk	27.5	7.49

¹ Recalculated for completely oxygenated blood at pH drawn.² The morning following ingestion of 3 pounds of steak.

TABLE 5
Urinary findings for various diet periods

SUBJECT	PERIOD	CONDITIONS	URINARY FINDINGS AS HOURLY RATE					pH
			Titratable acidity	NH ₃ -N	P.	SO ₃	N.	
			Gc. N/10	Mg.	Mg.	Mg.	Gm.	
Mc.	11:00 p.m. to 7:00 a.m. Mar. 25, 26	Fasting	28	40	59		1.17	5.1
	7:00 a.m. to 9:00 a.m. Mar. 26 ¹	One pound of lean steak at 7 a.m. at rest	16	32	70		1.49	6.5
	9:00 a.m. to 11:00 a.m. Mar. 26		17	37	66		1.54	6.0
	11:00 a.m. to 1:00 p.m. Mar. 26		27	35	57		1.76	5.3
Mc.	1:00 p.m. to 3:00 p.m. Mar. 26		23	22	45		0.85	5.0
	10:00 p.m. to 7:00 a.m. Aug. 18, 19	Fasting	3	4	14	28	0.16	7.0
	7:00 a.m. to 11:00 a.m. Aug. 19	One pound of lean steak at 7 a.m. normal activity	12	7	64	96	0.73	7.5
	11:00 a.m. to 3:00 p.m. Aug. 19		13	18	51	122	0.53	6.5
Mc.	10:30 p.m. to 7:00 a.m. Aug. 22, 23	Fasting	24	28	49	92	0.59	5.3
	7:00 a.m. to 11:00 a.m. Aug. 23	One quart of milk at 7 a.m. normal activity	7	13	36	80	0.80	7.5
	11:00 a.m. to 3:00 p.m. Aug. 23		13	17	43	75	0.65	6.3
	7:30 a.m. to 9:30 a.m. Feb. 18	One pound of steak at 7:30 a.m. at rest	9	23	50			7.0
H.	9:30 a.m. to 11:30 a.m. Feb. 18		5	9	42			7.6
	11:30 a.m. to 1:30 p.m. Feb. 18		9	16	60			7.3
	1:30 p.m. to 3:30 p.m. Feb. 18		21	21	54			5.7
	9:00 a.m. to 11:30 a.m. Feb. 25	One pound of steak at 9 a.m. at rest	8	8	32			6.9
C.	11:30 a.m. to 1:30 p.m. Feb. 25		13	11	66			7.0
	1:30 p.m. to 3:30 p.m. Feb. 25		12	16	51			6.6
	3:30 p.m. to 4:30 p.m. Feb. 25		11	16	36			6.2
	6:00 a.m. to 8:45 a.m. Aug. 31	Fasting	21	28	42	85	0.46	5.2
B.	8:45 a.m. to 2:00 p.m. Aug. 31	One pound of steak at 8:45 a.m. normal activity	15	25	28	120	0.66	6.0
	2:00 p.m. to 4:45 p.m. Aug. 31		18	14	45	106	0.66	4.9

¹ Day following the ingestion of 3 pounds of steak.

for subject H. The fasting plasma bicarbonate value for subject M. was 4 volumes per cent higher following a night during which the urinary excretion was essentially acid (1361 cc. 0.1 N acid per 24 hours) than when the excretion was more nearly neutral (144 cc. 0.1 N acid per 24 hours). These results might at first sight appear to be at variance with those of Fitz and Van Slyke ('17) who found that urinary acidity as measured by titratable acidity and ammonia could be correlated with plasma CO_2 combining power, if concentration was also considered. For normal individuals the average error found by Fitz and Van Slyke was 3.2 volumes per cent, the maximum 7 volumes per cent. Van Slyke has emphasized that the correlation is only an approximation. The changes in plasma CO_2 observed in our studies were, in all instances, well within the error of the Fitz-Van Slyke determinations. Our results would indicate that the recent deductions of Saywell ('32) that raisins and figs produce a more alkaline body reaction because they produce a decrease in urinary acid are extremely hazardous. It appears to us that acid-base data for the blood should have been collected in the experiments of Saywell before deductions were drawn.

DISCUSSION

The equivalent in NaHCO_3 as alkaline ash of 1 pound of bananas is 2 gm. This foodstuff was included in the present studies because it does not contain the large amount of organic acid found in oranges. One quart of orange juice contains the alkaline ash equivalent of roughly 4 gm. of NaHCO_3 . Since the enormous amounts of citric acid present must show a temporary acid effect (oxidation is not quantitative), and since 4 gm. of NaHCO_3 only temporarily shifts the CO_2 combining power by 2 volumes per cent, one would, on theoretical grounds, expect no significant change in the acid-base equilibrium of the blood to the alkaline side following the ingestion of orange juice or, for that matter bananas. In Blatherwick's classic experiments on the orange ('22), it was shown that the tolerance of the body to citric acid oxidation was not ex-

ceeded. Slight increases in urinary alkalinity were observed, since the base excreted overshadowed the amount of organic acid not oxidized. Blatherwick concluded, "One may therefore feel secure in eating unlimited amounts of oranges without fear of acidotic effects." He was fully aware of the very slight excess of base over acid excreted.

The experiments with orange juice, bananas, and milk, while they offered no theoretical ground for an expected change in alkali reserve, serve as a basis of comparison for the acid ash food steak. In only one of the four individuals studied could a significant temporary lowering of the plasma BHCO_3 be demonstrated following the ingestion of a pound of steak. The question arises as to whether this effect should be ascribed to the liberation of acid ash in the catabolism of the food or to the re-absorption of acid following the alkaline tide. The latter explanation would appear probable since on the occasion of the maximum fall of plasma bicarbonate, the excretion of urinary acid, ammonia, nitrogen, and phosphorus was decreasing. That the effect is transient is demonstrated by the high pre-breakfast value found for the same individual following a day in which 3 pounds of steak were ingested, and confirmed by the data obtained for the individual who ate the equivalent amount of acid ash food for 2 days. A calculation of the maximum rate of acid excretion by the kidneys on the steak diet, as equivalents of ammonium chloride, yields only 0.3 gm. per hour. The equivalent of 1 pound of steak as ammonium chloride is approximately 3 gm. The liberation of the acid in the catabolism of the steak obviously covers a period exceeding 6 hours, as gauged by phosphorus, sulfur, and nitrogen excretions. Barring the added effect of re-absorption of acid following the alkaline tide, slow liberation of acid in the catabolism of the steak would in itself be insufficient to cause a significant change in the body alkalinity.

The results of the study indicate rather clearly that no significant change in the reaction of the blood (pH) following the ingestion of either acid or ash or alkaline ash foods is induced. The significance of the isolated observation of a

temporary fall in the plasma bicarbonate following the acid ash food in relation to a possible physiological effect remains to be considered. The fall, 3 m.Mol., is less than that induced by exercise and was not increased when normal activity was superimposed. Moreover, the absolute values for the low bicarbonate contents are well within the normal fasting range of the other individuals studied.

SUMMARY

1. The daily administration of 30 gm. of sodium citrate was the smallest amount of alkali which produced a shift in the acid-base equilibrium of the blood drawn before breakfast, outside the normal variation of the individual, but inside the normal variation of a normal group.

2. There was no significant difference in the acid-base picture of the blood of a normal individual drawn before breakfast whether the mixed diet consumed contained excessively acid or excessively alkaline ash foodstuffs. The diet regularly consumed by the individual contained potentially as much acid ash as a diet planned to be excessively acid. A diet containing 240, 204, and 93 gm. of protein on consecutive days and 200 cc. N acid equivalent of ash daily, produced a doubtfully significant shift in the blood picture to the acid side.

3. The ingestion of 1 quart of milk, 1 quart of orange juice or 1 pound of bananas produced no temporary shift in the plasma pH or alkali reserve.

4. The ingestion of 1 pound of steak temporarily lowered the plasma bicarbonate significantly in one of four individuals studied. The absolute values were well within the group range.

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SOME FACTORS INFLUENCING NITROGEN ECONOMY DURING PREGNANCY¹

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The phase of metabolism in human pregnancy which was among the earliest to be studied was that of nitrogen exchange, beginning with Zacharjewsky (1893). Since then some fifteen investigations have been reported from various laboratories and yet the information on this subject appears at times to be hopelessly confused, and our concept of the nitrogen needs of the human mother during pregnancy often seems anything but clear.

Only the recognition of the multiplicity of factors, both fetal and maternal, affecting nitrogen economy during pregnancy; the observation of nitrogen metabolism in conjunction with that of other elements, particularly phosphorus, and with a knowledge of the general adequacy of the diet as a whole, such as total calories, available vitamins and characteristic foodstuffs; together with data on larger numbers of subjects with known nutritional histories can effect an accurate solution of this problem. The material presented in this paper adds to the accumulation of evidence and serves to indicate some reasons why past findings have been so variable.

LITERATURE

Reviews of the significance of various phases of nitrogen metabolism during pregnancy are given by Harding ('25)

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² Mary Pemberton Nourse Memorial Fellow of the American Association of University Women, 1931-1932.

and more recently by Hunscher et al ('33). A summary of the nitrogen balance experiments during pregnancy which have been reported in the literature including the present paper have been compiled in table 1. This summary does not include the experiments by Sillevs ('04) and additional ones

TABLE 1
Summary of nitrogen balance studies during pregnancy

INVESTIGATOR AND REFERENCE	NUM- BER OF SUB- JECTS	NUM- BER OF BAL- ANCES	TOTAL DAYS	AVERAGE NITROGEN DAILY		COMMENT
				Intake	Storage	
				Gm.	Gm.	
Zacharjewsky (1893)	6	6	60	18.54	3.05	6 to 18 days antepar- tum. Limited diet
Schrader ('00)	2	2	11	22.37	6.36	4 to 7 days antepar- tum. Computed diet
Slemmons ('04)	4	4	39	14.89	2.39	2 sets twins. Diets computed
Hahl ('05)	2	7	41	18.00	2.04	17 to 24 days ante- partum
Bar ('05)	3	3	34	17.43	6.68	Low fecal nitrogen
Hoffström ('10)	1	23	161	12.90	1.84	Continuous observa- tion
Landsberg ('12)	6	13	53	16.10	2.50	
Landsberg ('14)	9	14	70	16.10	1.23	
Wilson ('16)	3	37	259	13.14	2.84	Continuous observa- tion. Computed diets
Sandiford et al. ('31)	1	5	50	13.88	0.30	Computed diets. Feces not analyzed
Macy et al. ('31)	1	2	15	11.80	2.55	Specified diet
Hunscher et al. ('33)	3	12	49	17.58	1.90	Pregnancy subsequent to heavy lactation
Coons-Blunt ('30)	9	23	116	11.03	2.15	Home diets
This paper	6	23	92	11.58	1.97	Home diets

from Bar ('07), for which the figures at the authors' disposal were only partially complete. The summary shows the number of observations and subjects and the average daily intake and storage found by the various investigators. All of these results are far from being comparable, but some of the differentiating characteristics have been listed under comments.

The workers prior to Hoffström concerned themselves with studies during the very last days of pregnancy and often used subjects requiring hospitalization for one reason or another. The case histories are often brief or entirely lacking. There is no record of a preliminary period or of only very short ones for accustoming the subjects to the changes in diet. Usually the diet was limited as to variety, and in one case it consisted of only milk, meat, bread, tea, and water (Zacharjewsky, 1893).

Another important source of experimental error, which extends also to some later studies, was the use of published analyses for computing the nitrogen content of the diets. Donelson and co-workers ('31) have shown that errors from this procedure may vary from 1 to 38 per cent, being more significant in the short time studies and arising apparently from differences in composition of the foodstuffs rather than from errors in calculation. Thus an error of only 5 per cent in the computation of a high protein diet would change the balance approximately 1 gm., representing from 30 to 100 per cent of the amount stored in most cases.

Records of very low fecal nitrogen, 0.2 to 0.5 gm. daily in some studies (Bar, '07; Slemmons, '04; Wilson, '16) indicate coefficients of digestibility for nitrogen around 95 to 99. Such figures suggest the possibility of improper preservation of collections, which, if true for urine, might easily account for very high retentions through losses of urinary nitrogen. Even the error from failure to analyze feces may equal or exceed a small balance, since some data (Coons, '30) show coefficients of digestibility as low as 70, leaving three times as much fecal nitrogen as the customary coefficient of 90 would allow. The entire series of twenty-three balances for nine women reported previously by one of us (table 3) showed an average coefficient of 83 or almost twice the expected amount of fecal nitrogen.

The chief difficulty in the way of comparing these nitrogen balances is the wide variation between the nitrogen consumption, around 18 to 20 gm. daily, of the subjects of the early

studies, and the 11 to 12 gm. daily of those of the more recent series. This confirms previous observations made in this laboratory (Coons, '32) that there is a tendency nowadays to habitually lower protein consumption, and finds support in the lower urinary nitrogen figures reported by Rowe and his associates ('30). It seems probable also that more of the nitrogen of these recent lower diets comes from vegetable or cereal proteins, and less of it from animal sources.

Exceptions to these records of low nitrogen consumption are two of Macy's women who had become accustomed to high intakes, as high as 28 to 29 gm. of nitrogen daily, in their performance of heavy lactation and had carried over some of this tendency into the subsequent gestation period.

On the whole, those studies (table 1) which cover most of pregnancy, namely Hoffström's work and all later ones, representing a total of thirty-nine women, showed a definite tendency to an average storage around 2 gm. of nitrogen daily. There is no evidence in the data available to indicate that retentions are at all constant throughout the whole of pregnancy, because actually wide and irregular fluctuations in storage occur even when the same individual is observed continuously for only a part of gestation. However, cause for individual retentions varying greatly from the above average should be sought in the quality and quantity of the diet, in the previous nutritional history of the mother during both the gravid and non-gravid states, or in some other unusual phenomena associated with the case.

EXPERIMENTAL

The data herein reported included twenty-three 4-day balance experiments during pregnancy and one 4 weeks prior to pregnancy on six women. The activities of these women have been described (unpublished data) and more complete case histories, including total weight changes, age, size of the baby, and success in lactation, are presented elsewhere (Coons, '33).

The manner of weighing the food and making collections was the same as that used in previous studies (Coons and

Blunt, '30; Coons, '30). A portable refrigerator was devised and used throughout the series as an extra precaution for preservation of excreta from the time of collection until removal from the home to the laboratory, since many of the metabolism periods were scheduled for warm months in a southern climate. This apparatus consisted of a double compartment, well-insulated fireless cooker, placed in the home, usually on a back porch, and cooled with ice cubes from an electric refrigerator. Rubber ice bags were used to hold the cubes and these were refilled by the investigator two to three times daily as needed. In this manner feces and urine were cooled rapidly from body temperature to about 40° to 50°F., remaining at this temperature until the next visit by the investigator when collections were removed to the laboratory and cared for at once. As customary in this work, toluol was also used in the bottles for urine collections.

Determinations of creatinine daily left no doubt as to the completeness of the collections of urine. Total nitrogen was determined daily on urine and also on the composite aliquot made up for mineral analyses. Analyses were made on the dried samples of food and feces, composited for the period. The macro-Kjeldahl method was employed throughout for determining total nitrogen.

RESULTS AND DISCUSSION

The nitrogen balances are shown in table 2, and table 3 summarizes some of the dietary habits in relation to the absorption of food.

From table 2 it will be seen that the intake varied widely, 8.08 to 19.13 gm. daily, and that although the retention varied somewhat, in fourteen instances it was between 1 and 2 gm. daily. Analyses for total energy content of the diet (Coons, '33) showed a range in intake from 36 to 58 calories per kilogram with an average of 44, and indicated no marked dietary deficiency in this respect. Differences in retention rates were apparently unrelated to any period of gestation, and tendencies to high storage were individualistic, exhibited only by cases II and VI.

The higher retentions of these cases were associated with higher levels of intake, a relatively larger proportion of milk, meat, and eggs in the diet (table 3) and larger amounts of the vitamin B complex. The extra supply of this last factor came from an ounce of Vitavose daily, the whole grain cereals

TABLE 2
Nitrogen retention by Oklahoma women during pregnancy

CASE AND PERIOD	WEEK OF PREGNANCY	NITROGEN INTAKE	NITROGEN OUTGO			BALANCE	NITROGEN PER KILO	
			Urine	Feces	Total		Intake	Retained
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
I	0 - 4	8.16	6.82	0.85	7.67	+ 0.49	0.17	0.010
	1 2	9.37	6.79	0.88	7.67	+ 1.70	0.19	0.030
	2 27	9.61	7.40	1.04	8.44	+ 1.17	0.17	0.021
	3 31	9.89	7.44	1.00	8.74	+ 1.15	0.17	0.019
	4 34	9.88	7.83	1.04	8.88	+ 1.00	0.16	0.016
	5 38	8.80	5.98	0.91	6.89	+ 1.91	0.14	0.030
II	1 26	11.84	8.13	1.26	9.39	+ 2.44	0.22	0.045
	2 30	14.26	9.13	1.35	10.48	+ 3.78	0.25	0.067
	3 34	14.62	9.92	1.60	11.52	+ 3.10	0.25	0.052
III	1 33	11.56	8.57	1.58	10.15	+ 1.40	0.16	0.020
	2 37	12.86	8.85	1.75	10.60	+ 2.26	0.18	0.031
IV	1 31	11.84	9.18	1.22	10.40	+ 1.44	0.14	0.017
	2 35	9.74	7.18	1.27	8.45	+ 1.29	0.11	0.015
	3 39	11.64	8.64	1.45	10.09	+ 1.56	0.13	0.017
V	1 28	10.08	7.38	1.02	8.40	+ 1.68	0.16	0.026
	2 33	8.08	6.02	1.33	7.35	+ 0.73	0.12	0.011
	3 38	10.01	5.85	1.34	7.19	+ 2.82	0.15	0.043
	4 39	8.97	6.61	1.25	7.86	+ 1.11	0.14	0.017
VI	1 18	17.70	12.46	2.07	14.53	+ 3.17	0.32	0.058
	2 23	19.13	11.99	1.94	13.93	+ 5.20	0.32	0.087
	3 27	13.54	10.59	1.50	12.09	+ 1.45	0.22	0.023
	4 31	12.01	8.12	2.04	10.16	+ 1.85	0.18	0.028
	5 35	11.96	7.64	1.61	9.25	+ 2.71	0.18	0.041
	6 38	9.90	7.31	1.57	8.85	+ 1.02	0.15	0.015

and liver regularly (unpublished data) for case II, and for case VI in the first two periods from much milk, liver, and whole grain cereals, the last two of which did not appear in the diets of the later studies with her. Experimental evidence of the relation of the vitamin B complex to nitrogen economy

has been presented by various investigators (Funk, '23; Macy et al., '30; Sherman and Derbigny, '32). It is impossible, however, from these experiments on freely self-chosen diets

TABLE 3

The character of the diet as related to the absorption of nitrogen

CASE AND PERIOD	WEIGHT	CHIEF PROTEIN-CONTAINING FOODS AVERAGE DAILY INTAKE				ABSORPTION				
		Meats	Liver	Eggs	Milk	Nitrogen coeff. of digesti- bility	Food daily (dry)	Feces daily (dry)	Food: feces	
	<i>Kilo</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Per cent</i>	<i>Gm.</i>	<i>Gm.</i>		
I	0 ¹	49.0				90	355	17	20	
	1 ¹	49.9				91	433	17	25	
	2	56.7	4	9	36	697	89	433	24	18
	3	59.0	31		39	536	90	461	21	22
	4	61.2	30		30	600	90	439	23	19
	5	63.5	21		22	527	90	482	21	23
II	1	54.4	34	17	56	593	89	538	26	20
	2	56.2	78	12	68	835	91	560	26	21
	3	59.4	62	19	61	726	89	560	31	18
III	1	71.2	144		42	178	86	518	36	14
	2	72.5	43		38	459	86	627	28	22
IV	1	86.6	115		11	329	90	575	27	21
	2	88.4	20		11	465	86	541	25	22
	3	90.7	46			649	88	705	31	23
V	1	63.5	30		60	209	90	499	18	28
	2	64.4	11		58	195	84	454	27	17
	3	65.3	57		19	249	87	519	26	20
	4	65.8	24		22	485	86	501	25	20
VI	1	54.9	30	48	68	1162	88	577	41	14
	2	60.0	36	21	50	1285	90	617	33	18
	3	62.6	31		44	796	89	542	26	21
	4	65.3	10		31	1008	83	553	39	14
	5	65.8			10	887	87	492	33	15
	6	66.2				957	84	465	29	16
Average:										
This series		39	21	35	628	88	530	28	20	
Chicago series		33	21	29	416	83	464	34	14	

¹ Record of foodstuffs not retained.

to estimate the relative importance of these dietary factors in their effect on nitrogen economy. Besides these, too many non-dietary influences were playing a part.

The gains in weight of the different subjects during the time covered by these balance studies were similar, around 2 kg. a month, except in cases III and V and in case VI during the last 2 months (table 2). However, there was no reason to believe that such gains were of similar body composition. The total gains from the prepregnant weight to that at the end of gestation showed greater differences in individuals and were more indicative of changes in nutritional status in the maternal organism itself.

For example, case IV, a tripara, some 30 pounds overweight according to commonly used standards, gained only 15 pounds during gestation and that during the last trimester. Case VI, some 20 pounds below weight at the beginning, gained a total of 35 pounds, 30 of it before the thirtieth week of pregnancy. If it be assumed that, in the instance of case VI, the maternal organism brought itself to a state of good nutrition during gestation, as indeed outward appearances and a feeling of well-being indicated, then a gain of only 15 pounds remained for the pregnancy proper. In like manner it was found that the other primiparae, cases I, II, and V, gained 15 to 20 pounds in excess of that needed for normal nutrition.

The fact that if excessive gains occurred they did so before the last trimester of pregnancy, the period for greatest fetal accretions, and evidence here and elsewhere (Coons and Blunt, '30; Hunscher et al., '33) that very high nitrogen retentions, when found, tended to occur early in pregnancy, emphasize the need for a knowledge of the nutritional history of the mother during all of gestation as well as during the pre-gestation period, in order to interpret the findings for nitrogen retention. In other words, it is futile to expect a given level or curve of nitrogen retention for all women during the course of a pregnancy, unless a normal, well-nourished maternal organism at the beginning, and the same state of nutrition without addition or subtraction at the end of gestation, is presupposed.

In the previous study lactation seemed related to nitrogen metabolism (Coons and Blunt, '30). It is of interest to add

here that in this series, cases III, IV, and VI were quite successful, case II had difficulty with inverted nipples, and case I, threatening failure at first, finally, after some 4 weeks with special attention to diet, rest and avoidance of worry, established full breast feeding which continued satisfactorily until weaning. On the other hand, case V failed completely, and the infant was receiving all artificial feedings by the sixth week. Both the diets and storages of nitrogen for cases I and V were the poorest of the series and are believed to represent borderline deficiencies for women of their nutritional status.

Attention is called to another interesting observation in this series of studies. From tables 1 and 3 it will be seen that the diets averaged slightly better in quantity and quality than did those of the Chicago series representing similar home conditions and technic of investigation. Despite an apparently poorer absorption in the earlier group the storage was slightly higher, averaging 2.15 compared to 1.97 gm. daily, or 19.5 to 17.0 per cent of the respective intakes. Omitting the storage figures for those periods when the diets contained unusual amounts of the vitamin B complex (case II) or were much above the customary intake (case VI, periods 1 and 2), the present group averaged a storage of only 1.57 gm. daily, compared to the 2.15 gm. for the northern group with similar nitrogen consumption.

A poorer absorption was shown for the Chicago group by the coefficients of digestibility for nitrogen, with the average of 83 equal to the lowest coefficient recorded in the present series, and likewise the average of 88 for this group equal to the highest recorded in the former study. It was shown also by the ratio of dry food to dry feces daily, representing crudely the high or low absorption of all foodstuffs, which ratios averaged 20 and 14 for the present and former series respectively. The diets of the southern group were characterized by larger amounts of vegetables and cereals (Coons, '33), as was indicated also by the greater dry weight for food composites daily (table 3). The more frequent use of laxa-

tives by these women was explained partly by these tendencies to lower fecal residues. Such tendencies were coincident with better calcium retention (unpublished data) and may have been referable to the increased sunlight. These facts serve to accent the poorer nitrogen retention in these Oklahoma women and point to some disturbed endogenous metabolism. The disturbance may have been due to a low quality of protein or a lack of some accessory factor and may be related to the lower basal metabolism reported for this section. At least it calls for further investigation.

In summarizing it would seem, however, that all the possible factors influencing nitrogen metabolism tend to operate during pregnancy. On the positive side are the impulses to replenish body reserves, to supply elements to a growing organism, and to provide for some vaguely defined but quantitatively large biological preparation for the losses of parturition and the puerperium and for the performance of lactation. Along with these come the increased desire for food, and consequent higher intake, the improved utilization, and the tendency to retain that absorbed or economize on protein catabolism, all a part of the normal events in any pregnancy. On the negative side, however, there may be operating one or more of such factors as the poor quality or limited quantity of protein available, a series of digestive disturbances such as nausea and 'heart-burn' common to pregnancy, or other pathological conditions necessitating a restriction of the amount of protein available for absorption. Doubtless other unknown conditions affect one or more phases of the cycle of protein metabolism. It is surprising that the maternal organism adjusts itself as well as it does to all these varied influences.

SUMMARY

Twenty-three 4-day balance experiments during pregnancy and one 4 weeks prior to pregnancy on six women are presented in this report.

The results show relatively lower nitrogen retentions than those of previous studies, except when the diets were increased with additional protein or supplemented with accessory factors, particularly the vitamin B complex. The average intake, including supplemented diets, was 11.58 gm. of nitrogen daily with a storage of 1.97 gm.

Tendencies to greater absorption rates as shown by higher coefficients of digestibility and by lower fecal residues were observed in this series and are discussed in relation to the metabolism of other elements.

A knowledge of the nutritional history of the mother, both in the pregravid period and through all of gravidity is essential to the proper interpretation of nitrogen retentions during pregnancy.

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THE IRON CONTENT OF FOODS USED IN A MUNICIPAL HOSPITAL

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In the course of a study of iron metabolism, in the metabolic ward of the Russell Sage Institute of Pathology, analyses of the iron content of the food, provided by the diet kitchen of Bellevue Hospital, were made throughout the experiment. Because of the fact that this food is representative of the diet served patients in a large municipal hospital, it is thought desirable to present the results obtained.

The specimens of food were collected over a period of 7 months and, with the exception of fruits contained in jars and fresh peaches, at least three samples of each specimen were studied. No two samples of the same food were analyzed at the same time. The subjects receiving the foods were allowed only distilled water or beverages prepared with distilled water. All their food was cooked in iron-free water. No iron pots or pans were used. The foods were prepared in enamel kitchenware and with heavily plated cutlery. The specimens for analysis were sent to the laboratory in glass bottles. All of the fresh vegetables were thoroughly washed with iron-free water before drying to insure freedom from soil. Foods which contained considerable moisture were dried in 100 gm. portions in porcelain casseroles on a steam bath. The dried foods were first weighed, then thoroughly ground in a porcelain mortar, passed through a fine copper mesh sieve and an aliquot portion was taken for analysis. The opening

TABLE 1
The iron content of foods (milligrams per 100 gm. E.P.) compared to values given in standard tables

NO.	FOOD	NUMBER OF SPEC- IMENS	MAXIMUM	MINIMUM	AVERAGE	P. & N. (28)	SHERMAN (32)	ROSS (29)	OTHER SOURCES
1	Apples, f	3	0.31	0.26	0.29	0.31	0.36	0.30	
2	Apricots, c.	3	1.02	0.68	0.86		0.60		
3	Apricots, d.	3	7.25	6.45	6.74	7.26		1.40	
4	Asparagus, c.	3	1.20	0.44	0.73		1.00 (f.)	1.00 (f.)	1.70 (f.), L, C & A
5	Bacon, f.	3	0.94	0.67	0.80		1.50	1.30	
6	Bananas, f.	3	0.60	0.35	0.46	1.76	0.64	0.60	
	Beans, lima, c.	3	2.20	2.09	2.16	11.66 (f.)	2.40 (f.)	2.00 (f.)	
					2.16			7.00 (d.)	
7	Beans, string, c.	3	1.20	0.80	0.96				
	Beans, string, f.	3	1.05	0.63	0.91		1.00	1.10	0.38 L & F
8	Beef, lean	3	2.70	1.83	2.22	0.93	3.00	2.40	3.33 L & F
9	Bread, white	3	1.38	1.18	1.27		0.90	0.85	0.67 L & F
	Bread, whole wheat	3	1.97	1.88	1.93		1.60	1.65	
10	Butter, f.	3	0.26	0.17	0.20		0.20	0.20	
11	Cabbage, f.	3	0.64	0.38	0.53	0.34	0.43	1.10	
12	Carrots, f.	3	0.64	0.43	0.54	1.07	0.64	0.60	0.51 L & F
13	Cauliflower, f.	3	1.09	0.95	1.02	1.43	0.94	0.60	
14	Celery, f.	3	0.80	0.35	0.51	0.77	0.62	0.50	
15	Cheese, American	3	0.89	0.64	0.79	1.38	1.30	1.30	
16	Cherries, c.	3	1.38	0.93	1.18	0.48 (f.)	0.40 (f.)	0.40 (f.)	
	Cherries, jar	1			0.81				
17	Chicken, boiled	3	2.73	2.34	2.51	1.01 (d.)		3.20	
						0.70 (l.)	2.70	2.70	
18	Cocoa, A.P.	6	19.00	8.00	12.64	3.13			
	Cocoa, bean without shell	3	3.55	3.33	3.43				
	Cocoa, shell alone	3	57.10	22.86	35.90				
19	Coffee, whole bean	3	7.02	6.16	6.66				
	Coffee, ext. from 100 gm.	3	1.13	0.79	0.91				
20	Corn, c.	3	0.57	0.35	0.43	2.60 (f.)	0.47 (s.)	0.80 (g.)	
21	Corn flakes	5	4.92	1.64	2.98	2.78	2.90 (sd.)	0.90 (meal)	
22	Cranberries, f.	3	0.47	0.40	0.45		0.22	0.60	
23	Cream, 20 per cent	3	0.33	0.15	0.23		0.22	0.20	
24	Egg, whole	3	2.90	2.03	2.38	2.52	3.00	3.00	1.92 cooked L & F
25	Egg yolk	3	6.86	6.35	6.52	7.60	8.60	8.60	
26	Farina	3	1.23	0.89	1.02		0.80		
27	Fish, salmon, c.	3	0.99	0.80	0.87	0.83 (f.)		0.80 (l.)	
28	Fish, tuna, c.	3	2.12	1.50	1.80			1.20 (f.)	
29	Flour, white	3	2.00	1.33	1.73			1.20 (1.4 in oil)	
30	Grapes, Malaga, f.	3	0.71	0.54	0.64	0.91 (p.)	1.00	1.00	
31	Grapefruit, f.	3	0.23	0.21	0.24	0.27	0.73	0.30	
						0.27	0.27	0.30	

of the cans, in the case of tinned food, presented a source of iron contamination. This was overcome by carefully washing the foods with distilled water as they were removed from the tins. Whenever washing was not permissible, as in the case of canned corn and fish, a specimen was collected from the center of the food, using a silver spoon to scoop out enough material for duplicate analyses, avoiding the food around the edges of the can. The canned fruits, however, were very difficult to dry, due to the large amounts of sugar present. The question of getting a representative aliquot portion was surmounted by using practically all of the partially dried fruit for ashing. Cereals, dried fruits and materials low in water content were ashed directly in porcelain crucibles.

In all cases where appreciable differences were obtained for a particular foodstuff from those given in standard tables or from those found upon previous examination, duplicate analyses were made. In all such tests good checks were obtained. The method for the determination of iron was the Elvehjem ('30) modification of the Kennedy ('27) procedure.

The results of the analyses and comparisons with values in some standard tables are given in table 1. Certain facts are apparent from these figures. In the first place, some of the foods served in this municipal hospital dietary vary rather markedly in iron content from those of the standard tables of Sherman ('32), Rose ('29), Leichsenring and Flor ('32), and Levine and co-workers ('32). The most marked differences are: bacon [0.8],¹ lean beef [2.2], chicken [2.5], cocoa [12.6], whole egg [2.3], egg yolk [6.0], farina [1.02], tuna fish [1.9], flour [1.7], liver [5.6], oatmeal [5.1], fresh peas [1.4], green peppers [0.69], potatoes [0.68], dried prunes [3.57], fresh spinach [4.4], walnuts [3.8]. Of these seventeen items all except three are in the high iron-containing class; four are meats and four fresh vegetables.

Those foods which agree closely in iron content with the standard tables are: fresh string beans [0.96], butter [0.204],

¹ The values given in brackets are those found in our determinations in milligram Fe per 100 gm. of edible portion.

corn flakes [2.98], cranberries [0.448], cream [0.233], grapefruit [0.24], milk [0.21], fresh pears [0.32], rice [0.987], fresh tomatoes [0.443], fresh turnips [0.49]. Of these eleven foods one is a high iron-containing food, two contain a moderate amount of iron and the rest are low in iron content. Three

TABLE 2

The variation of maximum values from minimum values of iron content of different specimens of the same foods

WITHIN 25 PER CENT	26 TO 50 PER CENT	51 TO 100 PER CENT	100 PER CENT OR MORE
Apples, fresh	Bacon, fresh	Apricots, c.	Asparagus, c.
Apricots, dried	Beans, string, c.	Bananas, f.	Celery, f.
Beans, lima, c.	Beef, lean	Beans, string, f.	Cocoa, A.P.
Bread, white	Carrots, f.	Butter, f.	Cocoa shell alone
Bread, whole wheat	Cheese, American	Cabbage, f.	Corn flakes
Cauliflower, f.	Cherries, c.	Corn, c.	Cream, 20 per cent
Chicken, boiled	Coffee percolated from 100 gm.	Flour, white	Milk, f.
Cocoa bean without shell	Egg, whole	Lettuce, heart	Olive oil
Coffee, whole	Farina	Macaroni	Orange, whole
Cranberries, f.	Fish, tuna, c.	Orange juice	Peaches, c.
Egg, yolk	Grapes, Malaga, f.	Pears, c.	Peppers, green
Fish, salmon, c.	Grapefruit, f.	Pears, f.	Prunes, dried
Lamb, f.	Lettuce, leaf	Spinach, f.	Salt, table
Liver, f.	Oatmeal	Sugar, granulated	Tomatoes, c.
Onions, f.	Peas, f.	Tea, extract from 100 gm.	
Peaches, f	Pineapple, c.		
Peas, c.	Pineapple, f.		
Potatoes, f.	Rice, white		
Tea leaf, whole	Tomatoes, f.		
Yeast, dried	Turnips, f.		
	Walnuts		

of the foods which approximate in iron value those given in standard tables are fresh vegetables, two are fruits (fresh), and three are milk products.

It is of interest to note that of the thirteen canned foods examined, cherries [1.183], tuna fish [1.797], peaches [1.08], pears [0.653], pineapple [1.39], tomatoes [1.08], six in all, have a much higher iron content than they show in the fresh

state. Single determinations of four fruits in jars, cherries, peaches, pineapple and pears were made, and in each case iron values were obtained which were higher than those in the fresh state, but lower than in the canned condition.

Table 2 illustrates the variation in iron values of the same food when analyses are made on portions taken from supplies given to the patients after an interim of several months. Twenty foods show less than 25 per cent variation between maximum and minimum values; twenty-one between 26 and 50 per cent; fifteen between 51 and 100 per cent, and fourteen more than 100 per cent.

There is no special class of food which shows any marked consistency in the relationship between maximum and minimum values except possibly meats, fish and eggs in which less variation is obtained than in the case of milk, cream and butter.

One of the important factors to be considered in arranging a diet is not so much the number of milligrams of iron in 100 gm. of the edible portion of food, but the actual amount of iron ingested when the weight of an average portion is taken into consideration. Table 3 shows the absolute quantity of iron in average portions and table 4 gives a list of foods arranged in order of iron content per 100 gm. of edible portion and in order of iron content per average portion. When the weight of an average portion of food is taken into consideration, such high iron-containing foods as cocoa, walnuts, corn flakes, flour, macaroni and lettuce leaf afford relatively little iron in the ordinary diet.

CONCLUSIONS

1. Two hundred twenty-six samples of fifty-five common foodstuffs used in a municipal hospital were analyzed for iron content and considerable variation was found in different samples of the same food.

2. Seven of thirteen canned foods which were analyzed contained more iron than in the fresh state, probably due to concentration in the process of canning and possibly due to iron contamination from the can.

TABLE 3

Actual average iron intake (weight of average portion multiplied by per cent of iron)

FOOD	WEIGHT OF AVERAGE PORTION IN GRAMS	AVERAGE IRON CONTENT MILLI- GRAM PER 100 GM.	TOTAL IRON PER AVERAGE PORTION IN MILLIGRAM
Apples, f.	110	0.29	0.36
Apricots, dried	40	6.74	2.70
Apricots, c.	100	0.86	0.86
Asparagus, c.	100	0.73	0.73
Bacon, f.	40	0.80	0.32
Bananas, f.	100	0.46	0.46
Beans, lima, c.	50	2.16	1.08
Beans, string, c.	100	0.96	0.96
Beans, string, f.	100	0.91	0.91
Beef, lean	80	2.22	1.72
Bread, white	65	1.27	0.76
Bread, whole wheat	65	1.93	1.16
Butter	30	0.20	0.06
Cabbage, f.	100	0.53	0.53
Carrots	100	0.54	0.54
Cauliflower, f.	125	1.02	1.28
Celery, f.	50	0.51	0.26
Cheese, American	25	0.79	0.20
Cherries, c.	100	1.18	1.18
Chicken, boiled	100	2.51	2.51
Cocoa, ground, A.P.	5	12.64	0.63
Coffee, percolated from 12.5 gm.	12.5	0.91	0.11
Corn, c.	100	0.43	0.43
Corn flakes	25	2.98	0.75
Cranberries, f.	30	0.45	0.13
Cream, 20 per cent	30	0.23	0.07
Egg, whole	60	2.38	1.43
Egg yolk	30	6.52	1.96
Farina	25	1.02	0.26
Fish, salmon, c.	65	0.87	0.57
Fish, tuna, c.	100	1.80	1.80
Flour, white	5	1.73	0.09
Grapes, Malaga, f.	100	0.64	0.64
Grapefruit, f.	100	0.24	0.24
Lamb, f.	100	2.51	2.51
Lettuce, heart	35	0.50	0.18
Lettuce, leaf	35	1.04	0.36
Liver, f.	80	5.66	4.53
Macaroni	60	1.45	0.87
Milk, f.	200	0.21	0.42
Oatmeal	30	5.10	1.53
Olive oil	5	0.18	0.01
Onions, f.	100	0.44	0.44
Orange juice	100	0.27	0.27
Orange, whole	100	0.39	0.39
Peaches, c.	100	1.08	1.08
Peaches, f.	100	0.54	0.54
Pears, f.	110	0.32	0.36
Pears, c.	100	0.65	0.65
Peas, c.	100	0.89	0.89
Peas, f.	100	1.44	1.44
Peppers, green	100	0.69	0.69
Pineapple, f.	100	0.32	0.32
Pineapple, c.	100	1.39	1.39
Potatoes	100	0.69	0.69
Prunes, dried	50	3.57	1.79
Rice	45	0.99	0.44
Spinach, f.	100	4.40	4.40
Sugar, granulated	35	0.09	0.03
Tea extract from 4 gm.	4	0.72	0.03
Tomatoes, f.	100	0.44	0.44
Tomatoes, c.	150	1.08	1.62
Turnips, f.	100	0.49	0.49
Walnuts	10	3.81	0.38

TABLE 4

FOODS ARRANGED ACCORDING TO ABSOLUTE IRON CONTENT (MILLIGRAMS PER 100 GM. E.P.)		FOODS ARRANGED ACCORDING TO IRON VALUES WHEN AVERAGE INTAKE OF PORTION IS CONSIDERED (MILLIGRAMS)	
Cocoa shell alone	35.90	Liver, fresh	4.53
Tea leaf, whole	15.46	Spinach, fresh	4.40
Yeast, dried	14.56	Apricots, dried	2.70
Cocoa, A.P.	12.64	Chicken, boiled	2.51
Apricots, dried	6.74	Lamb, fresh	2.51
Coffee, whole, ground	6.66	Egg yolk	1.96
Egg yolk	6.52	Fish, tuna, canned	1.80
Liver, fresh	5.66	Prunes, dried	1.79
Oatmeal	5.10	Beef, lean	1.72
Spinach, fresh	4.40	Tomatoes, canned	1.62
Walnuts	3.81	Oatmeal	1.53
Prunes, dried	3.57	Peas, fresh	1.44
Cocoa bean without shell	3.43	Egg, whole	1.43
Corn flakes	2.98	Pineapple, canned	1.39
Chicken, boiled	2.51	Cauliflower, fresh	1.28
Lamb, fresh	2.51	Cherries, canned	1.18
Egg, whole	2.38	Bread, whole wheat	1.16
Beef, lean	2.22	Beans, lima, canned	1.08
Beans, lima, canned	2.16	Peaches, canned	1.08
Bread, whole wheat	1.93	Beans, string, canned	0.96
Fish, tuna, canned	1.80	Beans, string, fresh	0.91
Flour, white	1.73	Peas, canned	0.89
Macaroni	1.45	Macaroni	0.87
Peas, fresh	1.44	Apricots, canned	0.86
Pineapple, canned	1.39	Bread, white	0.76
Bread, white	1.27	Corn flakes	0.75
Cherries, canned	1.18	Asparagus, canned	0.73
Peaches, canned	1.08	Peppers, green	0.69
Tomatoes, canned	1.08	Potatoes	0.69
Lettuce, leaf	1.04	Pears, canned	0.65
Cauliflower, fresh	1.02	Grapes, Malaga, fresh	0.64
Farina	1.02	Cocoa, A.P.	0.63
Rice, white	0.99	Fish, salmon, canned	0.57
Beans, string, canned	0.96	Peaches, fresh	0.54
Coffee percolated from 100 gm.	0.96	Carrots, fresh	0.54
Peaches, jar	0.96	Cabbage, fresh	0.53
Beans, string, fresh	0.91	Turnips, fresh	0.49
Peas, canned	0.89	Bananas, fresh	0.46
Fish, salmon, canned	0.87	Rice, white	0.44
Apricots, canned	0.86	Onion, fresh	0.44
Cherries, jar	0.81	Tomatoes, fresh	0.44
Bacon, fresh	0.80	Corn, canned	0.43
Cheese, American	0.79	Milk	0.42
Asparagus, canned	0.73	Orange, whole	0.39
Tea, extract from 100 gm.	0.72	Walnuts	0.38
Peppers, green	0.69	Lettuce, leaf	0.36
Potatoes, fresh	0.69	Pears, fresh	0.36
Pears, canned	0.65	Apples, fresh	0.36
Grapes, Malaga, fresh	0.64	Pineapples, fresh	0.32
Peaches, fresh	0.54	Bacon, fresh	0.32
Carrots, fresh	0.54	Orange juice	0.27
Pineapple, jar	0.53	Celery, fresh	0.26
Cabbage, fresh	0.53	Farina	0.26
Celery, fresh	0.51	Grapefruit, fresh	0.24
Lettuce, heart	0.50	Lettuce, heart	0.18

TABLE 4—Continued

FOODS ARRANGED ACCORDING TO ABSOLUTE IRON CONTENT (MILLIGRAMS PER 100 GM. E.P.)		FOODS ARRANGED ACCORDING TO IRON VALUES WHEN AVERAGE INTAKE OF PORTION IS CONSIDERED (MILLIGRAMS)	
Turnips, fresh	0.49	Cheese, American	0.17
Bananas, fresh	0.46	Cranberries, fresh	0.13
Cranberries, fresh	0.45	Coffee, extract from 12.5 gm.	0.11
Onions, fresh	0.44	Flour, white	0.09
Tomatoes, fresh	0.44	Cream, 20 per cent	0.07
Corn, canned	0.43	Butter	0.06
Orange, whole	0.39	Sugar	0.03
Pears, jar	0.37	Tea, extract from 4 gm.	0.03
Pineapple, fresh	0.32	Olive oil	0.01
Pears, fresh	0.32		
Apples, fresh	0.29		
Orange juice	0.27		
Grapefruit, fresh	0.24		
Cream, 20 per cent	0.23		
Milk, fresh	0.21		
Butter, fresh	0.20		
Olive oil	0.18		
Salt, table	0.11		
Sugar, granulated	0.09		

3. In determining the iron content of diets it is important to take into consideration the weight of an average portion of food as well as the percentage of iron.

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PROTEIN, CALCIUM AND PHOSPHORUS INTAKES OF COLLEGE WOMEN AS INDICATED BY NITROGEN, CALCIUM AND PHOSPHORUS OUTPUTS ¹

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Accepted figures are available for the amounts of protein and minerals thought desirable in the human diet, and various methods are used to learn how actual dietaries compare with these standards. Group dietary studies yield valuable information, but do not show the actual intake of each subject. On the other hand, accurate individual weighed dietary studies can seldom be conducted without interfering in some way with free choice of food by the subjects. Another method, which can be used for adult subjects eating their usual freely chosen diets, is the determination of output as an indication of dietary intake. Unless the intake is at a very low level, the normal adult tends to adjust nitrogen and mineral metabolism to his supply, so that determinations of the amounts eliminated indicate the amounts contained in the diet. For the work here described, the protein, calcium and phosphorus intakes of twenty-five college women were studied in this manner.

Healthy college women, graduate and undergraduate students, served as subjects for 4 days in the fall and again for 4 days in the winter, each making complete collections of urine and feces. Subjects were weighed daily during the experimental periods, and the average weights for each subject for both periods (table 1) were available for use in making

¹ Contribution No. 17 Department of Home Economics.

calculations. The subjects were asked to follow their customary habits, including between-meal eating. In most cases they were not informed as to the exact nature of the experiments so that spontaneous choice of food might not be affected.

TABLE 1
Weights of subjects used

	WEIGHT IN KILOGRAMS	
	Fall period	Winter period
A	49.0	49.9
B	73.5	73.9
C	56.2	55.8
D	54.4	56.2
E	49.9	50.8
F	58.1	59.0
G	52.2	54.9
H	55.3	56.2
I	57.6	59.4
J	70.3	68.5
K	51.7	53.0
L	56.7	58.5
M	57.2	59.0
N	57.6	63.0
O	56.7	58.1
P	56.2	57.2
Q	58.1	59.4
R	48.1	55.8
S	58.5	58.5
T	42.6	43.1
U	60.3	62.1
V	55.3	55.8
W	51.7	51.7
X	54.0	54.0
Y	62.1	64.4
Average	56.1	57.5

Full lists of foods eaten were kept by the subjects. In no case did a period include a holiday or special occasion when meals would likely vary from the normal and every effort was made to secure samples at a time when students were eating in their customary manner.

The urine for each day was measured and samples preserved and the feces for each period were dried and prepared for analysis in the usual manner. Nitrogen determinations were made on daily samples of urine for all subjects, using the Kjeldahl quantitative method. Quantitative determinations on urine and feces for each period for each subject were made for calcium according to the volumetric method of McCrudden ('11) and for phosphorus according to the volumetric method of the Association of Official Agricultural Chemists ('25). All determinations were made in duplicate.

The amount of average daily urinary nitrogen was calculated for each subject for both periods. Protein ($N \times 6.25$) used per day was calculated, using the commonly accepted assumption that approximately 10 per cent of the food protein is lost in the feces. Average daily calcium and phosphorus outputs were calculated for each subject for each period from determinations on urine and feces. These figures, to be used as indicative of nitrogen (or protein), calcium and phosphorus intakes, were calculated to the common basis of 70 kg. of body weight so that comparisons could be made.

Chittenden ('32) summarizing from his extensive investigations concerning the minimum protein requirements of the human body, concludes "that man may apparently adjust himself to a lowered intake of protein food, with a protein metabolism corresponding to 0.12 gram of nitrogen per kilogram of body-weight." This corresponds to 53 gm. of protein per 70 kg. per day. Using the best data now available, Sherman ('32) finds the average for normal adult maintenance to be about 0.5 gm. protein per kilogram of body weight per day. While the Sherman minimum requirement is, on this basis, 35 gm. of protein per 70 kg. per day; the suggested standard for adults, allowing a desirable margin for safety, is 1 gm. of protein per kilogram of body weight or 70 gm. of protein per 70 kg. per day. Similarly, data calculated to a uniform basis of 70 kg. of body weight have given the Sherman ('20 b) calcium requirements of 0.45 gm. per day and the Sherman ('20 a) phosphorus requirement of 0.88 gm. per

day. Sherman dietary standards ('32) per 70 kg. per day are 50 per cent above the minimum requirements, that is, 0.68 gm. calcium and 1.32 gm. phosphorus. The data secured from the twenty-five subjects, eating their customary diets, have been arranged in table 2, so that results from various subjects may be compared with each other and also with accepted standards for the adult human diet, the average daily figures for protein, calcium and phosphorus used per day, as calculated from output, being taken as indicative of the dietary intake.

Results for protein are distinctly different from those for calcium and phosphorus. Values equal to or above the Sherman standard of 1 gm. of protein per kilogram of body weight were found for only seven subjects in the fall and for three subjects in the winter. While no subject showed amounts below the Sherman requirements, six in the fall and four in the winter were below the Chittenden minimum. A different distribution was found for the figures for calcium and also for phosphorus, in every case a majority being above the Sherman standard. It is interesting to note that the calcium figures showed that more than half the subjects were using at least 1 gm. of calcium per day, the amount advocated for children by Sherman and Hawley ('22). In the whole series, one subject only showed a figure below the Sherman requirements, that being for calcium in the fall period.

While the figures for the Sherman standards and the Sherman requirements for calcium and phosphorus were calculated on the basis of 70 kg. of body weight, they are sometimes used as amounts per person per day. This is particularly true in discussions of dietary practices for women, since it is felt that the female should be provided with a generous rather than a scanty supply of calcium and phosphorus. Therefore, counts were made to determine the distribution of subjects on the basis of protein, calcium and phosphorus used per subject per day. This distribution shown as percentage of the number of subjects, is compared in table 3 with the distribution when the count is made on the basis of 70 kg. of body

TABLE 2

Distribution of subjects according to average amounts of protein, calcium and phosphorus used per day calculated to uniform basis of 70 kg. of body weight

FALL PERIOD						WINTER PERIOD					
Protein		Calcium		Phosphorus		Protein		Calcium		Phosphorus	
	Grams daily		Grams daily		Grams daily		Grams daily		Grams daily		Grams daily
		R	1.84					F	1.78		
		N	1.59					E	1.71		
		D	1.49					R	1.52		
		F	1.37					H	1.49		
		O	1.25					T	1.47	E	2.14
		I	1.20					W	1.42	T	1.67
		M	1.18					B	1.41	F	1.66
		Y	1.18					O	1.33	O	1.63
		K	1.14	D	1.96			K	1.32	I	1.53
		X	1.12	X	1.78			C	1.21	X	1.49
		W	1.10	E	1.77			M	1.07	H	1.48
		E	1.07	R	1.75			L	1.06	R	1.44
		A	1.06	L	1.68			I	1.05	B	1.42
		P	0.97	I	1.58			N	1.05	C	1.42
		G	0.93	O	1.58			X	1.02	M	1.42
E	99.0	V	0.93	K	1.49			D	1.01	K	1.41
L	77.9	U	0.89	Y	1.49			Q	1.00	Q	1.39
Y	77.5	Q	0.87	A	1.43			G	0.94	U	1.38
T	75.8	L	0.85	N	1.43			P	0.87	W	1.37
X	74.9	H	0.84	G	1.39	T	90.8	A	0.78	G	1.36
I	74.4	B	0.74	W	1.35	E	84.3	J	0.77	L	1.36
S	70.7	O	0.70	V	1.34	I	78.8	U	0.69	D	1.33

Sherman standards, per 70 kg. of body weight

	70.0		0.68		1.32		70.0		0.68		1.32
K	69.5	T	0.66	T	1.28	G	68.6	V	0.67	A	1.29
P	66.8	J	0.54	H	1.27	R	65.9	Y	0.63	J	1.22
Q	63.1			P	1.27	Q	64.7	S	0.50	P	1.21
A	62.9			B	1.25	P	62.1			Y	1.21
R	62.4			J	1.23	K	61.7			N	1.16
H	60.3			M	1.22	M	61.1			V	1.08
V	58.8			U	1.16	L	60.9			S	0.97
W	58.8			Q	1.14	A	60.5				
D	58.7			F	1.04	H	59.3				
U	58.7			S	1.02	Y	58.7				
O	58.6			C	0.96	X	57.8				
G	57.8					D	57.6				
						N	56.6				
						F	55.6				
						V	55.6				
						U	54.9				
						W	54.4				
						O	52.5				
	... ¹				 ¹				
M	51.0					B	51.6				
J	50.8					C	49.4				
B	47.8					S	47.0				
F	47.6					J	46.2				
C	46.0										
N	39.9										

Sherman requirements, per 70 kg. of body weight

	35.0		0.45		0.88		35.0		0.45		0.88
		S	0.44								
Aver.	62.8		1.04		1.39		60.7		1.11		1.40

¹ Chittenden minimum, 0.12 gm. of nitrogen per kilogram of body weight.

weight per day. By either method of counting, figures for many subjects are below the desired standards. Although values for no subjects fall below the protein requirement, the fact that more than two-thirds of them are below the protein standard may be a matter of some concern. While a good majority of the subjects have figures above the calcium and phosphorus standards per 70 kg. of body weight per day, it should be noted that some are below the requirements, particularly when considered per subject per day.

TABLE 3

Percentage distribution of subjects according to amounts of protein, calcium and phosphorus used

PER CENT OF SUBJECTS	PER SUBJECT PER DAY						PER 70 KG. PER DAY					
	Fall			Winter			Fall			Winter		
	Protein	Ca	P	Protein	Ca	P	Protein	Ca	P	Protein	Ca	P
Above Sherman standards ¹	28	84	12	12	80	20	28	88	56	12	88	72
Below Sherman standards, but above Sherman requirements	72	8	72	88	16	72	72	8	44	88	12	28
Below Sherman requirements ²	00	8	16	00	4	8	00	4	00	00	00	00

¹One gm. of protein per kilogram of body weight per day, 0.68 gm. calcium, 1.32 gm. phosphorus.

²One-half gm. protein per kilogram of body weight per day, 0.45 gm. calcium, 0.88 gm. phosphorus.

Relationships between the figures have been studied. In general, it seems that a subject showing low figures in one place is likely to have low figures elsewhere and a subject listed at the top of one column is apt to be high in others. Subjects having adequate supplies of calcium are likely to have good amounts of phosphorus, and vice versa, the coefficients of correlation between the amounts of calcium and phosphorus being 0.94 (a nearly perfect correlation) for the fall period and 0.78 (a good correlation) for the winter period. Some direct correlation also exists between amounts of protein and of phosphorus, the coefficients of correlation being 0.55 for the fall period and 0.61 for the winter period. No direct

correlation should be demonstrated between protein and calcium used.

Figures for each subject were used to study differences between the two periods. Of the twenty-five subjects, ten had more protein per 70 kg. in the fall, fourteen had more calcium and eleven had more phosphorus. Averages for protein, calcium and phosphorus per 70 kg. for the twenty-five subjects for the fall period are similar to those for the winter period. Whether studied subject by subject or on the basis of averages, no significant differences were found between the fall and winter periods.

Daily food lists which had been kept by the subjects were checked in an effort to find relationships between foods consumed and the protein, calcium and phosphorus used. These lists proved less helpful than anticipated, no doubt on account of variation in estimation of amounts. Counts of high calcium and phosphorus foods, especially milk and cheese, were found to correspond fairly well with the calcium and phosphorus used. The liberal use of milk, with correspondingly generous supplies of calcium and phosphorus, may perhaps be accounted for by the fact that all the subjects had had college courses in foods and nutrition and had learned of the value of milk in the diet.

In the original selection of subjects for this study, an effort was made to have some who ate freely chosen meals, as in cafeterias, and some who ate set meals, as in the college dormitory under college supervision or in organized houses. When the data were studied, only negligible differences were found between the average amounts of protein, calcium and phosphorus used per 70 kg. per day by the group eating freely chosen meals and the group eating set meals.

SUMMARY

Nitrogen, calcium and phosphorus outputs of twenty-five college women, eating freely chosen diets, have been determined to indicate protein, calcium and phosphorus intakes, using two periods of 4 days each.

In no case were daily protein figures below the Sherman requirement, but a majority were below the Sherman standard of 70 gm. of protein per 70 kg. of body weight. Protein per 70 kg. per day averaged 62.8 gm. for the fall period and 60.7 gm. for the winter period.

Calcium per 70 kg. per day averaged well above the Sherman standard of 0.68 gm., namely, 1.04 and 1.11 gm. for the fall and winter periods, respectively. Similarly, phosphorus per 70 kg. per day averaged 1.39 and 1.40 gm. for the two periods, both figures being above the Sherman standard of 1.32 gm.

Figures for protein used by the twenty-five subjects give averages distinctly below accepted standards, suggesting that the majority of the subjects were consuming less protein than is often advised. While averages for calcium and phosphorus were above the accepted standards, figures for some subjects were below accepted standards, in one case the calcium figure falling below the calcium requirement. Because some of the subjects were below the desired standards, it would seem a matter of concern to include sufficient high calcium and high phosphorus foods in the diet.

Whether studied subject by subject or on the basis of averages, no significant seasonal variations were found.

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FURTHER EXPERIMENTS WITH CATARACT IN ALBINO RATS RESULTING FROM THE WITHDRAWAL OF VITAMIN G (B_2) FROM THE DIET

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TWO FIGURES

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In a former paper (Day, Langston and O'Brien, '31) the authors described the occurrence of cataract in albino rats receiving a diet deficient in vitamin G (B_2). In a series of thirty-seven rats receiving the deficient diet, thirty-five developed cataract, the average time of appearance being the eighty-second day on the diet. Since that report, additional cases of cataract have developed in animals receiving the deficient diet. Cataracts have also been developed in animals under three other dietary regimes, in which the vitamin G was completely or only partially withheld. This lesion has appeared with such regularity that it can hardly be an isolated or accidental phenomenon. Not only the regularity of appearance, but also the specific nature of the lesion recommends it as a criterion of the vitamin deficiency. A dermatitis has been observed by all investigators working with vitamin G (B_2), but the manifestations of the condition vary widely. The dermatitis may appear as a loss of hair, as a roughening of the fur, as a mild erythema, or as distinct scabby ulcers on the face, back of the head, body, or legs. How much of the alopecia and ulceration is due to scratching is a matter of question. A stomatitis and salivation are frequently observed, but are by no means constant symptoms. On the other hand, the appearance of cataract has been found to be unmistakable and quite uniform. With very little experience it

can be observed with the naked eye in albino rats. With the ophthalmoscope it can, of course, be observed earlier and with greater precision. Since the preparation of this manuscript for publication, the authors' earlier work showing that cataract results from G-avitaminosis has been confirmed by O'Brien ('32) and Yudkin ('33).

EXPERIMENTAL

The animals used were of known dietary history, coming from stock which had received Sherman's diet 13 ('24) *ad libitum* and frequent feedings of fresh meat and lettuce for several generations. The young were separated from their mothers at about 21 days of age, or when they weighed 35 to 45 gm. To prevent coprophagy, they were kept in metal cages with raised floors of $\frac{3}{8}$ inch hardware cloth. The diets used were in all cases modifications of the Sherman and Spohn vitamin B complex deficient diet ('23), differing from each other chiefly in the manner in which the antineuritic vitamin was supplied. Diet 554 is the diet used by Bourquin and Sherman ('31) in their technic for the determination of vitamin G, the vitamin B (B_1) being supplied by an 80 per cent alcoholic extract of ground whole wheat. Diet 625 differs from it only in that the vitamin B is supplied by an 80 per cent alcoholic extract of rice polish. This extract is prepared as follows: 500 gm. of rice polish are stirred with 1500 cc. of 80 per cent (by weight) alcohol for a half hour or longer, allowed to stand overnight, again stirred for a half hour and filtered on a Büchner funnel. The rice polish is washed with 500 cc. of the 80 per cent alcohol in several small successive portions, and the filtrate and washings combined and evaporated at room temperature in front of an electric fan. This extract is finally evaporated down on cornstarch and the starch so prepared incorporated in the diet.

In diet 615, the vitamin B is supplied by the rice polish extract, but 40 per cent of the cornstarch is replaced by sucrose. This diet was used because Leader ('30) found that the presence of cane sugar in the diet increased the suscepti-

bility of rats to dermatitis. Diet 635 contains 25 per cent ground whole wheat, which supplies the necessary vitamin B, but also contains a limited quantity of vitamin G. This diet is essentially the same as that used by Sherman and Axtmayer ('27), Hunt ('28), and others. The exact compositions of these diets are summarized in table 1.

Diets 554, 615, and 625 are all deficient in, and possibly entirely free of, vitamin G. Young rats given these diets gained in weight rather rapidly for the first 2 weeks, more slowly

TABLE 1
Composition of vitamin G-deficient diets

	BOURQUIN-SHERMAN DIET 554	DIET 615	DIET 625	DIET 635
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Casein ¹	18	18	18	18
Salt mixture ²	4	4	4	4
Butter fat	8	8	8	8
Cod liver oil	2	2	2	2
Cornstarch	68	28	68	43
Sucrose	..	40
Whole wheat	25
80 per cent alcoholic extract of whole wheat, equivalent to	50
80 per cent alcoholic extract of rice polish, equivalent to	..	25	25	..

¹ Extracted with 60 per cent alcohol according to the method of Sherman and Spohn ('23).

² Osborne and Mendel salt mixture ('19).

for the next 2 or 3 weeks, remained nearly constant in weight for some time, then gradually declined in weight until death; the weight at death usually being greater than the initial weight (fig. 1). The first symptom to appear was usually alopecia, the hair frequently starting to come out during the second or third week and often leaving the animal quite denuded. Some litters showed no loss of hair at all, however. Later, scabby ulcers sometimes appeared on various parts of the body, and salivation and stomatitis were frequently observed. During the seventh or eighth week a conjunctivitis

and keratitis, with or without corneal vascularity, accompanied by a loss of hair around the eyes, were usually seen. These signs were followed in a short time by a dullness of the eyeball which later developed into an unmistakable opacity (fig. 2).

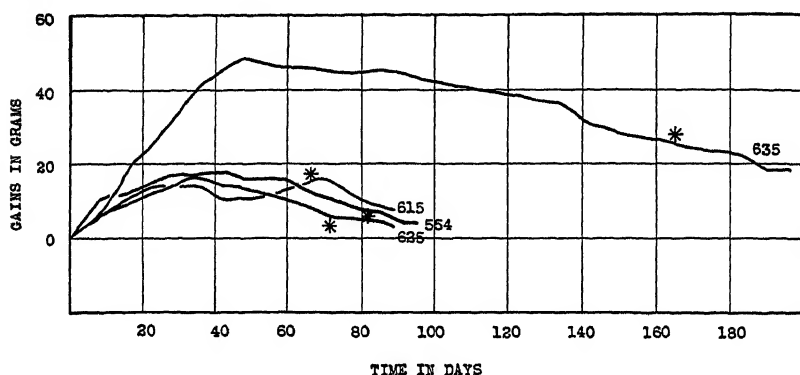


Fig.1 Average growth curves of animals receiving four diets deficient in vitamin G (B_2). The diet number is indicated at the end of each curve, and the asterisk marks the average time of appearance of cataract.

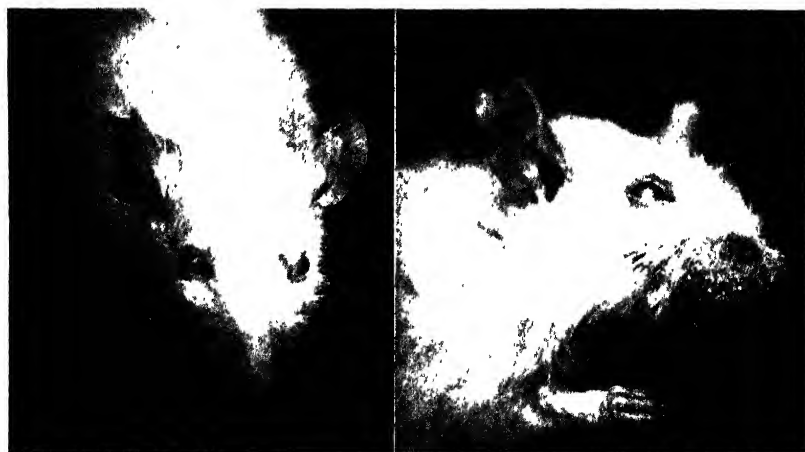


Fig.2 Photographs showing bilateral cataract resulting from a deficiency of vitamin G (B_2). This rat developed unmistakable cataract on the fifty-second day on diet 615, and was photographed on the ninety-third day. In addition to cataract, this animal showed a conjunctivitis and keratitis, stomatitis, salivation, epistaxis, and a dermatitis with ulceration on the abdomen, but no extensive alopecia.

As diet 635 contains a small amount of vitamin G, growth was more prolonged, the maximum increment in weight correspondingly greater, and the average survival period longer. These data are represented graphically in the figure, and are also given in table 2 together with other significant data.

TABLE 2

Summary of data concerning the incidence of cataract, weight increase, and survival period of albino rats receiving four diets deficient in vitamin G

DIET NO.	NUMBER OF RATS	CATARACT			AVERAGE MAXIMUM INCREASE IN WEIGHT		AVERAGE SURVIVAL
		Number	Incidence	Average time of appearance			
			<i>Per cent</i>	<i>Days</i>	<i>Grams</i>	<i>Days</i>	<i>Days</i>
554	2	2	100	82	18	42	96
615	4	4	100	67	16	49	...
625	72	70	97	72	16	35	86
635	5	5	100	165	50	71	198

DISCUSSION

Of the seventy-two rats receiving diet 625, seventy developed cataract between the fortieth and the eighty-seventh day on the diet, the average time of appearance being the seventy-second day. The incidence was thus 97 per cent. In compiling the data, only those animals that lived until the minimum time of appearance of cataract were included; those animals which died or were killed before that time were arbitrarily excluded from the tabulation. For this diet the average maximum increase in weight was 16 gm., and the average time required for the rats to attain the maximum weight was 35 days. The average survival period for rats in this group was 86 days, this figure representing the average of somewhat fewer than seventy-two animals, as many were chloroformed for histological section or for other reasons and were therefore not included in the calculations.

Both of the animals given the Bourquin and Sherman diet 554 developed cataract, as did all four of the rats given the diet containing 40 per cent sucrose (diet 615), the incidence of

cataract for these diets being 100 per cent, therefore. The time of appearance of cataract, the rate of growth, and the survival period were essentially the same for these groups as for the animals receiving diet 625. As they appeared to respond in every way exactly like the animals receiving diet 625, there seemed no necessity for increasing the number of cases.

With diet 635 growth was more rapid and continued longer, and the survival period was correspondingly greater, as would be expected because of the presence of vitamin G in the ground whole wheat present in the diet. Also, the cataracts appeared later and at a similar point on the growth curve, as is evident from an examination of the chart where the average time for the appearance of cataract is indicated by the asterisk. It appears that the factor responsible for the greater growth and longer survival in diet 635 also delayed the onset of the lens opacity. In fact, the ratio of days of growth to days for appearance of cataract is approximately the same for diets 625 and 635, although the absolute number of days of growth and days for appearance of cataract differ greatly. Similarly, the ratio of days survival to days for appearance of cataract is almost exactly the same for the two diets. That is, the growth factor and the cataract-preventive factor appear to be the same, or if not the same are present in like physiological amounts. It is significant that all of the five animals receiving diet 635 developed cataract. The presence of a small amount of a hypothetical cataract-preventive factor delayed for a time the appearance of the lesion, but was not able to prevent its ultimate development.

Diagnosis of cataract was made in every case by simple gross observations with the naked eye. Dilatation of the pupil was not necessary in the albino rats, because of the absence of iris pigmentation. This diagnosis of cataract was confirmed by ophthalmoscopic observations and histological section in enough cases to dispel any doubt as to the accuracy of diagnosis. Routine examinations with the ophthalmoscope were not made in all cases in the series reported in this paper. Had such observations been made, the time reported for the

appearance of cataract would have been materially shortened, as early changes in the lens can be observed with the ophthalmoscope, while the cataract must be rather far advanced to be evident in gross observation. Also, it is possible that the two animals which received diet 625 and are reported as not developing cataract, might have had early changes which would have been discernible with the ophthalmoscope, but which were not seen without the use of such an instrument. These two animals devoured the viscera of cage mates which died of the deficiency, and therefore obtained enough vitamin G to delay the onset of cataract.

Ample evidence has been accumulated in this laboratory and elsewhere to indicate that the diets used in these experiments contain an abundance of the antineuritic vitamin, or at least an adequate amount for much greater growth than was obtained. As the development of cataract was prevented in numerous cases by the feeding of autoclaved yeast, it appears that the cataract-preventive factor is a heat-stable vitamin. Whether it is identical with the dermatitis-preventive factor (P-P, vitamin G or B₂), or whether it is one of the other factors present in the vitamin B complex is a question that cannot be answered at the present time. Inasmuch as the lens of the eye has its embryological origin as an invagination of the body wall ectoderm and therefore comes from the same germ layer as the skin, it is not unreasonable to believe that a deficiency which results in pathological changes in the skin might also result in pathological changes in the lens of the eye, even though these tissues are apparently so unlike.

The accumulated evidence for the existence of factors in addition to the six commonly recognized vitamins has been largely based on growth (Harris, '32). Undoubtedly the new factors reported from some laboratories are identical with those reported from others, but because of the different methods of experimentation used it is difficult to correlate this mass of evidence. As growth is not specific, it would seem to the authors that one of the most useful methods in clarifying the situation would be to establish a definite pathology

with each deficiency, wherever this is possible. This pathological condition could then be recognized by other investigators, and the various lines of work thus correlated. The polyneuritis of vitamin B (B_1) deficiency is easily recognized, the dermatitis of vitamin G (B_2) deficiency has been quite commonly observed, and the symptoms accompanying a deficiency of Reader's vitamin B_4 have been described, but the pathological conditions resulting from deficiencies of the other postulated factors are less well known. It is possible that cataract is a manifestation of lack of one of these new factors, or, as seems more probable now, it is another manifestation of vitamin G (B_2) deficiency. If the latter is true, we have another and perhaps better criterion of vitamin G deficiency. The term 'dermatitis' is in itself indefinite, and as was pointed out in the introduction, the lesions produced by withdrawal of vitamin G are not specific and vary widely in different animals. Even with histo-pathological sections of the skin it is difficult to establish any very characteristic pathology as a result of the deficiency. The crystalline lens of the eye, on the other hand, is a transparent tissue that can be readily observed without injury to the animal, and in which even early changes are definite and unmistakable.

Salmon, Hays, and Guerrant ('28) showed that the presence of certain bacteria is an etiological factor in the development of the ulceration of vitamin G dermatitis. It is probable that scratching and other external irritations also contribute to the ulceration and alopecia. With these factors contributing to the development of the dermatitis, it is not surprising that there is considerable variation in the appearance of the signs of the deficiency. The position of the lens of the eye, however, would operate against its being affected by such external influences in experimental animals. Bacterial invasion could hardly be a factor in the development of the cataract; since the lens of the eye is non-vascular, even blood-borne organisms could not affect it directly. With a systemic disturbance as the only probable cause of the cataract it would appear that, theoretically at least, cataract should be a more reliable criterion of the deficiency than dermatitis.

SUMMARY

Cataracts have developed in nearly 100 per cent of the albino rats given diets freed of vitamin G (B_2). Cataracts also developed in all the animals given a diet containing 25 per cent whole wheat and therefore containing a limited quantity of vitamin G. With this latter diet, however, the cataracts appeared at a later date, and the time of their appearance can be correlated with the greater growth and longer survival of this group of animals. It thus appears that in the diets used the growth-limiting and cataract-preventive factors are identical, or else these diets are similarly deficient in both.

Cataract is suggested as a better criterion of vitamin G deficiency than dermatitis, as its appearance is more consistent and unmistakable, and also as its development is not influenced by external irritations and bacterial invasion which are undoubtedly contributing factors in the development of dermatitis.

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RELATION BETWEEN DEHYDRATION AND THE INSENSIBLE LOSS OF WATER¹

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In earlier papers we presented evidence to support our conviction that the heat production for periods of 24 hours or longer may be satisfactorily calculated from a knowledge of the amount of water vaporizing during the period. At that time, various sources of error were studied and discussed. We neglected, however, to deal experimentally with possible shifts in the insensible water due to differences in the water content of the organism.

It was already known that extreme depletion of the body's water would diminish the vaporization of water from the skin and lungs. Under these unusual circumstances, the method would no longer be valid. On the other hand, it was assumed that the moderate increases or decreases in the organism's content of water, such as might be commonly encountered, would not change the rate at which heat was being removed by vaporization of water.

But a group of investigators (Manchester, Husted and McQuarrie, '31) have recently come to the conclusion that changes in the degree of hydration are a much more important source of error than we had supposed. We have tried to emphasize in our earlier papers (Newburgh, Wiley and Lashmet, '31) the importance of calculating the heat production from the insensible loss of water, not from the insensible loss

¹ The expenses of this investigation were defrayed, in part, by a fund for the study of nutrition, created by Mr. W. K. Kellogg, of the Kellogg Corn Flake Company, Battle Creek, Michigan.

of weight, since the vaporized water may be as little as 82 per cent, or as much as 104 per cent of the insensible loss of weight, depending on the nature and amounts of the materials oxidized. The investigators just referred to did not, however, make the comparison in this way, even though the metabolic mixture of a single subject changed greatly from the foreperiod to that of dehydration. They were of necessity, then, dealing with at least two variables and totally ignoring one of them. We accordingly felt it necessary to investigate this question.

The subject was an apparently normal, highly educated young man (age 23 years, weight 60 kg., height 180 cm.), who understood the nature of the problem and who honestly tried to cooperate with us in every way. Two groups of experiments were carried out. The first group consisted of five consecutive periods. Throughout these studies, that occupied about 6 months, the subject lived in the hospital room especially designed for the quantitative study of metabolic problems. He was not confined to bed, but occupied himself for the most part by studying and writing. He was permitted to leave his room to go to the shower and telephone, and selected visitors were allowed. He played cards at times with one of the internes. The range of his activity is manifested by the variation in his heat production (calculated from his insensible loss of water) during the 53 days when his water intake was inadequate. His outgoing Calories varied from 2330 to 3008. All the weighings and the preparation of all the food were carried out by one of us.

The subject was fed a uniform diet selected for its dryness. In each period, he first received an adequate supply of drinking water and then various degrees of dehydration were produced by limiting this supply. The weight of the insensible water was determined by the method fully described by us (Newburgh, Wiley and Lashmet, '31). In general, less water was vaporized from the lungs and skin during the periods of dehydration than during the control periods. There was, however, no constant relationship between the degree of dehydra-

tion and the fall in the insensible loss of water. It may be seen from table 1 that the largest dehydration was accompanied by the largest insensible water.

TABLE 1
Summary of preliminary experiments

EXP. NO.	NUMBER DAYS	BODY WATER LOST	AVERAGE INSENSIBLE WATER	MINIMAL INSENSIBLE WATER
		Gm.	Gm.	Gm.
2 (a)	3	769	903	886
5	3	1525	874	796
4	4	1888	889	836
2 (b)	9	2267	844	718
1	4	2624	749	661
3	4	3227	985	935

Further, it was evident to both the subject and to us that the discomfort produced by the curtailment of water led him to omit many of his accustomed activities. For example, we noted that he was lying on his bed the greater part of the day. This was especially true in the first experiment. He gave up writing entirely during these periods. The average insensible loss of water—827 gm.—during 39 days of dehydration as opposed to 1082 gm., the average for 53 control days, may easily be accounted for by lessened activity. If the percentage of heat lost by the vaporization of water remained the same, such a decrease would represent a fall in heat production from about 2600 Calories to about 2000 Calories.

With these considerations in mind, we repeated the experiment under somewhat different conditions. The same subject was again used. This time he occupied a quiet room in the laboratory away from all the noises and confusion of the ordinary hospital ward. He himself reported that he was much less restless and not disturbed by distractions under these new conditions. He was at times visited by his wife and young baby. Throughout the experiment he was fed the same diet every day. Its composition was as follows: protein (by analysis) 54 gm.; fat 147 gm.; carbohydrate 232 gm., that

yielded 2484 Calories.² The dry solids of this diet by actual determinations were 435 gm. Its water content varied from 392 to 426 gm.

The experiment extended over 18 days and consisted of four consecutive periods: 8 days, foreperiod; 4 days, dehydration; 3 days, recovery; 3 days, dehydration. During the foreperiod and the first dehydration, no salt was added to the diet. Its low ash content is indicated by the low chloride excretion of the foreperiod, which averaged 0.55 gm. Cl. daily. In the recovery period and the second dehydration 10 gm. of sodium chloride were ingested daily.³ This addition was made in order to obtain a greater depletion of body water. During the foreperiod the subject drank approximately 1250 gm. of water per day. Dehydration was effected in both cases by omitting all drinking water. The body water lost during the first period of dehydration was restored in the first day of recovery by allowing about 3000 gm. of drinking water. During the second and third days of recovery, the subject again received about 1250 gm. of drinking water daily.

The pertinent data are given in table 2. From this we have constructed table 3, which shows the relationship of the insensible loss of water to changes in body water. It will be seen that under these new conditions the subject was less active than in the earlier studies, and that the range of his activity was also less. It may further be noted that his activity decreased as he became adjusted to his hospital routine. The average 24 hourly insensible loss of water for the first 4 days was 835 gm., as compared with 766 gm. for the second 4 days. The average insensible loss of water for each of the subsequent three periods is as follows: First dehydration 782 gm.; recovery 771; second dehydration 748

²The actual foods fed were: Puffed rice 12 gm.; Uneda biscuits 70 gm.; butter (salt-free) 60 gm.; cream (40 per cent) 180 gm.; sugar 35 gm.; 'mints' 75 gm.; jelly 20 gm.; potato 150 gm.; canned peas drained 120 gm.; lettuce 50 gm.; walnut meats 20 gm.; lean beef 135 gm. To obtain a uniform preparation sufficient meat for the period was ground after being freed of its fat, and preserved by freezing.

³The sodium chloride was administered in gelatin capsules which contained 0.25 gm. nitrogen.

gm. If one compares these values with the average for the second 4 days of the control period, it is obvious that no significant differences exist.

Since the subject pointed out that he was disinclined to exert himself during the periods of dehydration, a comparison may be made between quiet days of the foreperiod and those

TABLE 2

Data

DATE	AVAILABLE WATER			EXCRETED WATER		INSENSIBLE LOSS OF WEIGHT	
	In-gested	Pre-formed	By oxidation	Urine and stool	Insensible		
1932							
Oct.							
13	1677	— 6	255	816	844	935	Diet: Protein 54; fat 147; carbohydrate 232. Metabolic mixture: Protein 54; fat 83; carbohydrate 232. CO ₂ — O ₂ = 92
14	1647	— 6	255	1157	895	987	
15	1617	— 6	255	1117	840	932	
16	1674	— 6	255	872	763	855	
17	1674	— 6	255	1339	763	855	
18	1662	— 6	255	1144	708	800	
19	1654	— 6	255	1144	754	846	
20	1675	— 6	255	1266	840	932	
21	408	— 6	250	380	887	980	
22	394	— 6	250	374	765	858	
23	426	— 6	250	581	735	828	Diet: Same. Metabolic mixture: Protein 54; fat 83; carbohydrate 232. CO ₂ — O ₂ = 93 Diet: Same + 10 gm. NaCl + 1.3 gm. gelatin. Metabolic mixture: Protein 60.4; fat 74; carbohydrate 232. CO ₂ — O ₂ = 94.
24	410	— 6	250	340	740	833	
25	3434	+ 8	243	580	735	829	
26	1677	+ 8	243	861	787	881	
27	1702	+ 8	243	1441	790	884	
28	392	+ 8	243	1027	768	862	
29	421	+ 8	243	811	723	817	
30	404	+ 8	243	752	752	846	

of dehydration. For this purpose we selected from the control period the 4 days during which his activity was smallest. The average insensible loss of water for these days is 747 gm. With this may be compared the average of 746, for the last 3 days of the first dehydration, and 748 for the second dehydration. The uniformity of these three averages is striking.

Further, it should be pointed out that the lowest output of

insensible water (708 gm.) of the whole 18 days occurred on the sixth day of the control period, when the subject was receiving sufficient water.

TABLE 3

Relation between water content of body and insensible loss of water

DATE	BODY WATER ADDED OR LOST	INSENSIBLE WATER	TOTAL CALORIES ¹
Foreperiod. Drinking water about 1250 gm. daily. Low salt. Body water added, 150 gm.			
Oct., 1932			
13	+ 236	844	2042
14	— 156	895	2166
15	— 91	840	2033
16	+ 288	763	1846
17	— 179	763	1846
18	+ 59	708	1713
19	+ 35	754	1825
20	— 42	840	2033
First dehydration. No drinking water. Low salt. Body water lost, 2188 gm.			
21	— 615	877	2147
22	— 501	765	1851
23	— 646	735	1779
24	— 426	740	1791
Recovery. Drinking water 3000 gm. first day. Then 1250 gm. daily. High salt. Body water added, 2369 gm.			
25	+ 2370	735	1779
26	+ 280	787	1905
27	— 281	790	1912
Second dehydration. No drinking water. High salt. Body water lost, 2863 gm.			
28	— 1152	768	1859
29	— 862	723	1750
30	— 849	752	1820

¹ Calculated from the insensible water.

Finally if dehydration of the magnitude produced here disturbs the mechanism of dissipation of heat by vaporization of water, one would expect a decreasing insensible loss of water as dehydration increased. That this is not at all the case may be seen in table 2. Thus in the first dehydration

the insensible losses for the third and fourth days were respectively 735 and 740 gm. Likewise, in the second period of dehydration, the losses for the last 2 days were 723 and 752 gm.

We also produced a moderate degree of dehydration by the administration of ammonium chloride. The subject received 52 gm. in 4 days. The ingestion of water was the same as in the foreperiod. A loss of body water of only 1480 gm. resulted. The average loss of insensible water for the 4 days was 1243 gm., as compared with 1151 gm. for the control period. As a result of the ammonium chloride ingestion, the subject became restless and hyperpneic. The acidosis, as evidenced by the hypernea, must have resulted in an increased output of CO_2 . Since we were unable to measure the CO_2 output for 24 hours, we calculated the $\text{CO}_2 - \text{O}_2$ difference from the metabolic mixture in the usual way. This must have resulted in a value somewhat too low, and therefore its subtraction from the insensible loss gave a value for the insensible water which was correspondingly too high. This error together with the restlessness due to the nausea produced by the ammonium chloride may easily account for the recorded increase in insensible water. Because of these complications it is impossible to draw a sound conclusion regarding the effect of this type of dehydration on the insensible loss of water. At any rate, there is no evidence that the loss of body water effected in this way lowers the percentage of heat lost by the vaporization of water.

We have, then, obtained no support for the idea that anything less than extreme dehydration affects this percentage. Levine and Wyatt ('32) found that the basal insensible loss of weight of sixteen dehydrated infants was on the average 11 per cent below that of the same group after recovery. They have further observed that dehydrated infants studied in a respiration chamber continue to lose the normal percentage of heat by the vaporization of water, but that the average respiratory quotient is 0.76 as contrasted with the normal of 0.85. They calculate that this depression of the

quotient could account for 8 per cent of the decrease in insensible perspiration of the dehydrated infants. The remaining 3 per cent is so small a difference that it may be considered within the limits of error of the method employed. These results dealing with the basal state appear to be in agreement with those obtained by us for the 24-hourly cycle.

As mentioned earlier, our results are not in agreement with those obtained by Manchester, Husted and McQuarrie ('31). It is unfortunate that these authors did not deal with the insensible loss of water rather than the insensible perspiration, since they fed such a variety of diets. Careful examination of their charts reveals that the insensible loss sometimes rises and sometimes falls during dehydration. In the case of W.J., on the eighth day, water was added to the body, and on the ninth day body water was lost. Nevertheless, the insensible loss was much higher on the ninth than the eighth day. Another example may be seen in the case of J.R. On the twenty-first, twenty-second and twenty-third days there was progressive marked dehydration. Nevertheless, the insensible loss was highest on the twenty-third and least on the twenty-first.

The question of activity is not dealt with. Since our subject in the first series of experiments reduced his activity greatly when deprived of water, one is led to wonder if the children used in the investigation did not vary their activity sufficiently to account for some of the irregularities in insensible perspiration.

These investigators lay great emphasis on a comparison of heat production for 12-hour periods, measured by indirect calorimetry, with the predicted heat production estimated from the insensible perspiration by means of the Benedict and Root prediction table. In this way they obtain good agreement in their control periods, while in dehydration the heat production predicted from the insensible perspiration falls too low (table 1, Manchester, Husted and McQuarrie, '31).

In the first place, it is doubtful whether the total heat for a period of 12 hours can be calculated from a series of determinations occupying only a small fraction of every 2 hours. In the second place, we have previously pointed out that the calculation of heat production from the insensible perspiration may give highly erroneous results because of the effect of the respiratory quotients. Table 4 represents a recalculation of some of their data. The insensible water was obtained from the insensible perspiration by calculation of the CO_2 - O_2 difference of the diets used. It will be seen that in the

TABLE 4
Recalculation of table 1 (Manchester, Husted and McQuarrie)

INSENSIBLE PERSPIRATION	TOTAL CALORIES (12 HOURS)			CO_2 - O_2 CALCULATED FROM DIET	DEHYDRA- TION
	Predicted from insensible perspiration	Predicted from insensible water	Determined (Tissot)		
<i>Gm.</i>				<i>Gm.</i>	<i>Gm.</i>
326	657	765	659	-10	0
266	580	620	639	-10	- 914
319	650	748	630	-10	+ 259
322	654	799	672	+ 8	0
251	560	627	666	+ 8	-1308
293	614	682	693	-11	0
308	630	707	694	-11	+ 300
205	500	469	664	-11	-1233
220	518	506	655	-11	-2087
212	508	486	639	-11	-2752

case of the first control period, line 1 of the table, the apparent agreement between the heat determined by indirect calorimetry, viz. 659 Calories, and the heat predicted from the insensible perspiration, 657 Calories, disappears when the heat is calculated from the insensible water. It then becomes 765 Calories. Since in fact wide divergence occurs in the control periods, the significance of divergences in the dehydration periods are difficult to interpret.

Further, if dehydration lowers the percentage of heat lost by the vaporization of water, one would expect dehydration of the same degree to produce the same lowering of the insensible loss of weight in the case of a single subject. That this

did not occur may be seen from table 4, where two recorded dehydrations of 1308 and 1233 gm. resulted in insensible losses of weight of 251 gm. and 205 gm. respectively. This difference becomes even greater when it is expressed as the weight of the insensible water, viz., 259 gm. and 194 gm.

Accordingly, our very careful study of this paper does not convince us that dehydration to the extent effected here influences the percentage of heat removed by the vaporization of water.

CONCLUSION

The removal of approximately 6 per cent of the body water failed to alter the percentage of heat dissipated by the vaporization of water. Dehydration of this magnitude does not, therefore, introduce an error in the calculation of the total transformation of energy from the insensible loss of water.

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BEHAVIOR OF RATS OF DIFFERENT AGES ON A VITAMIN G DEFICIENT DIET

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FOUR FIGURES

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Although recognized for several years as an essential factor in good nutrition, vitamin G still presents many unknown phases. During the 16 years since Goldberger ('16) conducted his classical experiments which showed that pellagra in man is of dietary origin, little has been accomplished toward a completely satisfactory explanation of this disease. Attempts to produce pellagra experimentally in laboratory animals or to prove conclusively that vitamin G deficiency in rats is analogous to pellagra in humans have always furnished insufficient evidence to absolutely establish such correlations.

The histological work of Denton ('28) has led to the general acceptance that black-tongue in dogs and pellagra are of similar origin and nature. However, Gurin and Eddy ('31) report that the histological picture of rats suffering from vitamin G deficiency is different from the one seen in cases of human pellagra. On the other hand, Findlay ('28) describes 'pellagra-like' lesions occurring in rats deprived of vitamin G. Guha ('31) in discussing the etiology of pellagra points to the lack of clarity in evidence necessary for the acceptance of pellagra as the result of an uncomplicated deficiency of vitamin B₂ (G). Not infrequently the condition produced in rats on vitamin G deficient diets is referred to as pellagra. It is true that foods testing high in vitamin G

are usually found classified as good pellagra-preventive substances; and many of the symptoms, in so far as they have been compared, appear to be quite similar in the two deficiencies.

Goldberger and co-workers ('20) found an age as well as sexual and seasonal variation during a study of pellagrous patients in South Carolina. They noted that the disease was rare in children of 2 years or younger, and more prevalent in children between the ages of 2 and 10 years than in persons of 10 to 19 years, inclusive. Up to 20 years the incidence in both males and females was similar while between the ages of 20 and 54 years pellagra occurred many times more frequently in females than in males. There was a seasonal onset of the disease confined almost exclusively to the period from April to September with a well-defined peak in June.

Thatcher and co-workers ('31) report a seasonal variation in their rats on vitamin G deficient diets while Leader ('30) was unable to produce symptoms of vitamin G deficiency during December, January, and February. In reviewing the literature, no reference has been found to work which had as its purpose a study of the effect of insufficient amounts of vitamin G upon rats of different ages and sex.

EXPERIMENTAL

The experiments reported in these studies were carried out with the idea of obtaining some additional knowledge concerning the behavior of rats of different ages and sex on a diet deficient in vitamin G. Animals of 4, 6, 8, 10, and 12 weeks and 6, 9, 12, 15, and 18 months old were selected for study. Only a representative number of rats from a given litter were placed on the experimental diet at 4 weeks of age; the remaining were kept on the stock ration until they were 6 weeks old when a similar number were removed and so on until the litter was as completely distributed between the various age groups as was possible. In the case of the older animals it was impossible to adhere strictly to this method

of sampling. However, in every case the animals selected for study in any one group were representative of several litters. Table 1 gives the number of animals, distribution of sex and average weight of the members of each age group at the beginning of the test.

The basal diet used in this study was that described by Sherman and Spohn ('23) and had the following composition: casein, 18 per cent; cod-liver oil, 2 per cent; butter-fat, 8 per cent; Osborne and Mendel salt mixture, 4 per cent; and cornstarch, 68 per cent. This diet was supplemented with 0.25 gm. of rice polish which according to Munsell ('29)

TABLE 1

Number, age, average initial weight and sex distribution of the rats used

AGE OF ANIMALS	NUMBER OF ANIMALS	DISTRIBUTION OF SEX		AVERAGE INITIAL WEIGHT
				<i>Gm.</i>
4 weeks	8	4 M	4 F	57.3
6 weeks	8	4 M	4 F	85.0
8 weeks	8	4 M	4 F	131.5
10 weeks	8	4 M	4 F	164.6
12 weeks	8	4 M	4 F	180.9
6 months	8	4 M	4 F	290.8
9 months	4	2 M	2 F	290.8
12 months	6	3 M	3 F	317.2
15 months	8	4 M	4 F	288.5
18 months	8	4 M	4 F	326.6

furnishes a fair amount of vitamin B and a very small amount of vitamin G, thereby making vitamin G the first limiting factor. Consequently this means that all of the animals regardless of their initial weight or age received the same amount of vitamin G.

The criteria used for comparing the behavior of animals of different ages on this diet low in vitamin G were the ability of the younger rats to grow and of the older ones to maintain weight as well as the survival period of each group. At death, gross autopsy findings were noted.

The average change in weight for each age group over the entire survival period is shown in figure 1. Normal animals

4 weeks old when placed on this diet, slowly gained weight (about 20 gm. in all) during the first 3 months and thereafter throughout the remainder of the year maintained approximately this weight. Animals 6 weeks old maintained their initial weight with very little variation for more than a year. The rats in the other eight age groups lost weight from the start. Up to 6 months of age, the older the animals the more rapid was the decline in weight. Groups between 6 and 18 months of age showed very little difference in their growth responses on the vitamin G deficient diet. In no case was there a continued decline in weight throughout the experiment. Always, after reaching a certain low weight which was characteristic for each age group the animals continued for many months with relatively little variation in weight.

Since there was a considerable difference between the average weights of the animals of the different age groups at the time when each was placed on the experimental diet, it was questionable whether a fair comparison could be made between the actual changes in weight by the different groups. An animal weighing over 300 gm. could much better afford to lose 20 gm. than one weighing 75 gm. Consequently it seemed desirable to study and compare weight changes in terms of percentage of the initial weights of the animals. Calculations were made on the basis of average gains and not for each individual gain with subsequent averaging. These comparisons are shown in figure 2.

In every case up to 6 months of age, the older the rat the greater was the percentage loss of initial weight. The relationship between the age of the animals and their percentage weight loss was not a linear function, however. There were considerably greater differences in percentage losses between the groups of animals 4, 6, and 8 weeks old than between those 8, 10, and 12 weeks of age. In fact these latter groups showed very comparable percentage losses in weight.

Account was also taken of the fact that at the beginning of the experiment each of the younger rats possessed an innate impetus to grow, the amount of course depending upon

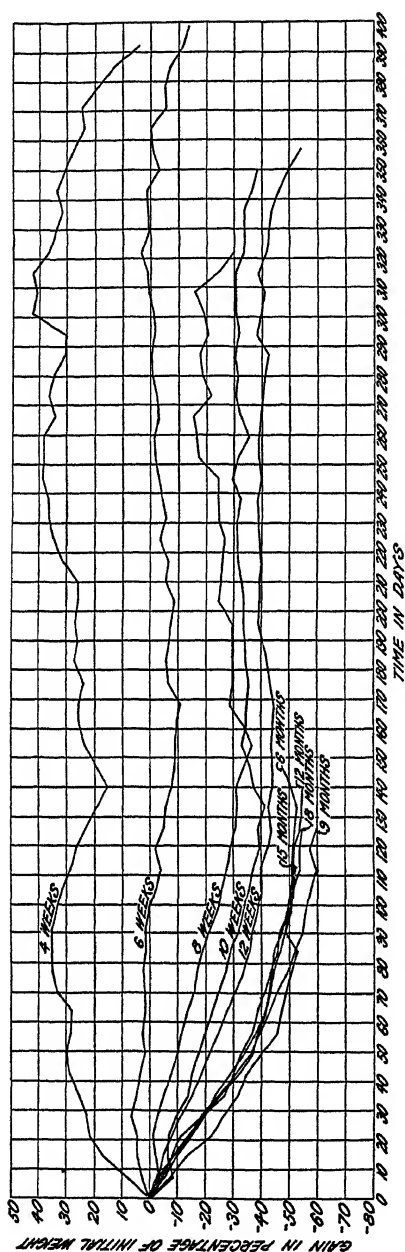


Fig. 2 Curves representing growth responses expressed as percentages of the initial weight of rats of different ages on a diet deficient in vitamin G. The age of each group of animals at the beginning of the experiment is shown along the curve for that group.

the age of the animal. In order to compare the behavior of the different age groups, the growth response of each was compared with that of normal rats of the same age and sex. The extent to which animals fell short of what could have been expected of them under normal conditions was then noted. Expressing normal growth by a base line, curves were plotted showing the average amount each group failed of reaching its normal weight. Such comparisons for male rats are shown in figure 3. The most outstanding point observed is that vitamin G insufficiency causes a great divergence from the normal growth of animals of all ages between 4 weeks and 18 months. By the end of the twelfth week, rats of the age groups under consideration showed an average weight that was between 157 and 218 gm. less than the weight normally expected.

Data relative to the length of survival show that older animals were unable to live for as long periods as those 3 months of age or younger. All of the rats died long before their normal life span had been covered. These data are incorporated in figure 1. It will be noticed that the survival behavior of all groups up to and including those 3 months of age were very similar. Twenty weeks had elapsed before at least one death had occurred in each of the younger groups of rats. The earliest death was during the sixteenth week in the group started at an age of 6 weeks. On the other hand, among the older animals the group of rats 15 months old at the beginning of the experiment suffered its first loss during the eighth week and all of these older groups had lost one animal by the twelfth week. The majority of the older animals were dead by the twentieth week.

From gross findings at autopsy it was concluded that the animals of all ages showed practically the same symptoms of vitamin G deficiency. Since all of the animals were subsisting on a diet which contained appreciable amounts of vitamin G, they did not develop as severe symptoms as rats which are given a diet totally devoid of this vitamin. In this respect the findings were more comparable with those occurring in

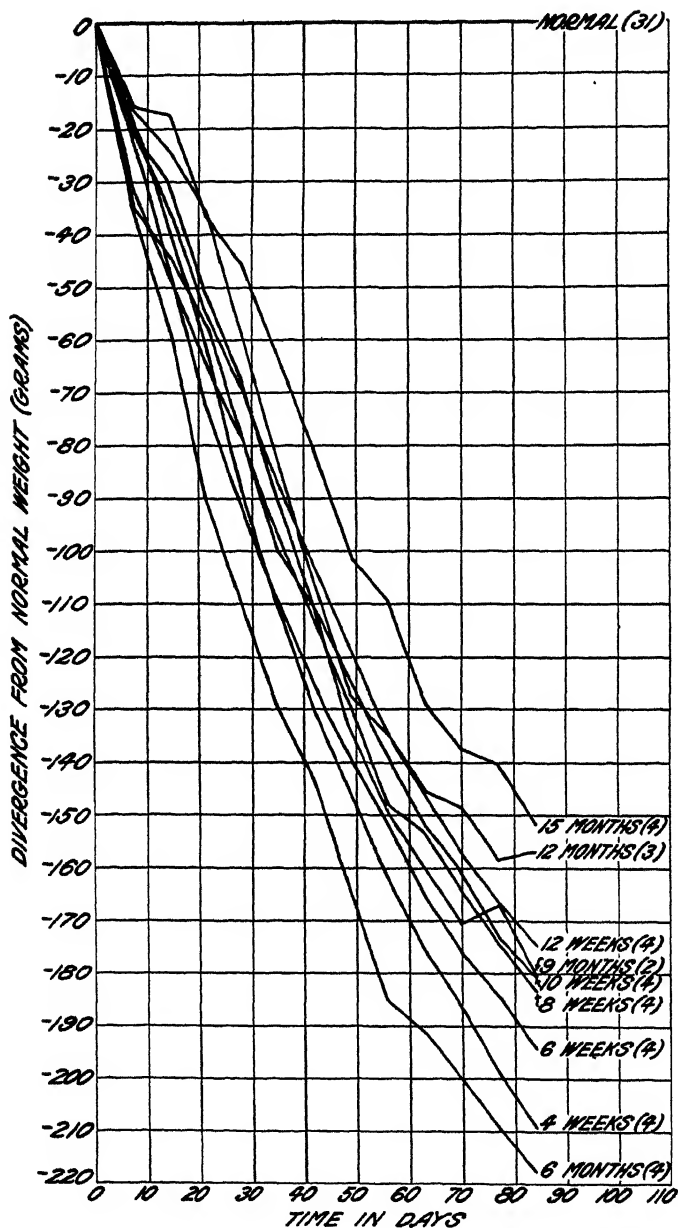


Fig. 3 Divergence from the normal gain in weight shown by male rats of different ages on a diet deficient in vitamin G. The age of the animals when put on the vitamin G deficient diet and the number in each group are indicated at the end of the curves.

cases of human dietary deficiencies where a vitamin is never entirely missing from the diet.

The usual development of the vitamin G deficient symptoms in the rats began with a loss in weight followed soon by a loss of hair and dermatitis. Lesions of various sizes appeared in a number of cases. These occurred more frequently about the head, shoulders and legs of the animal but might be found on any part of the body. They were not bilaterally symmetrical however. There was incontinence of the urine which was highly pigmented and in the later stages often bloody in appearance. Priapism occurred in almost every case. Upon autopsy a pathological condition of the gastrointestinal tract and liver was a marked characteristic of the disease. Generally, enormous hair balls were found in the stomach, and the walls of the cardiac portion were greatly thickened. Atrophy of the testes was usually observed. Except for a more pronounced tendency toward alopecia in the younger animals, there appeared to be no outstanding differences in the severity of the symptoms between the different age groups.

Comparing the responses of the males and females to vitamin G deficiency, among the younger animals, 4 and 6 weeks old, there was a tendency for the males to make slightly more rapid gains at first followed by a greater decline than that shown by the females. Both males and females of the 8-week-old group averaged almost an identical weight loss. In all of the older groups of animals 10 and 12 weeks and 6 to 18 months, the males suffered an increasingly greater actual loss in weight than the females (fig. 4). However, the fact must not be overlooked that male rats, especially the older ones, weighed much more at the beginning of the test than the females in the corresponding age groups. A comparison of weight changes based on the initial weight of the animals offers quite a different picture. Such a summary is given in table 2. Both males and females of the same age lost approximately the same percentage of their initial weight over a period of 16 weeks. This does not mean that animals in

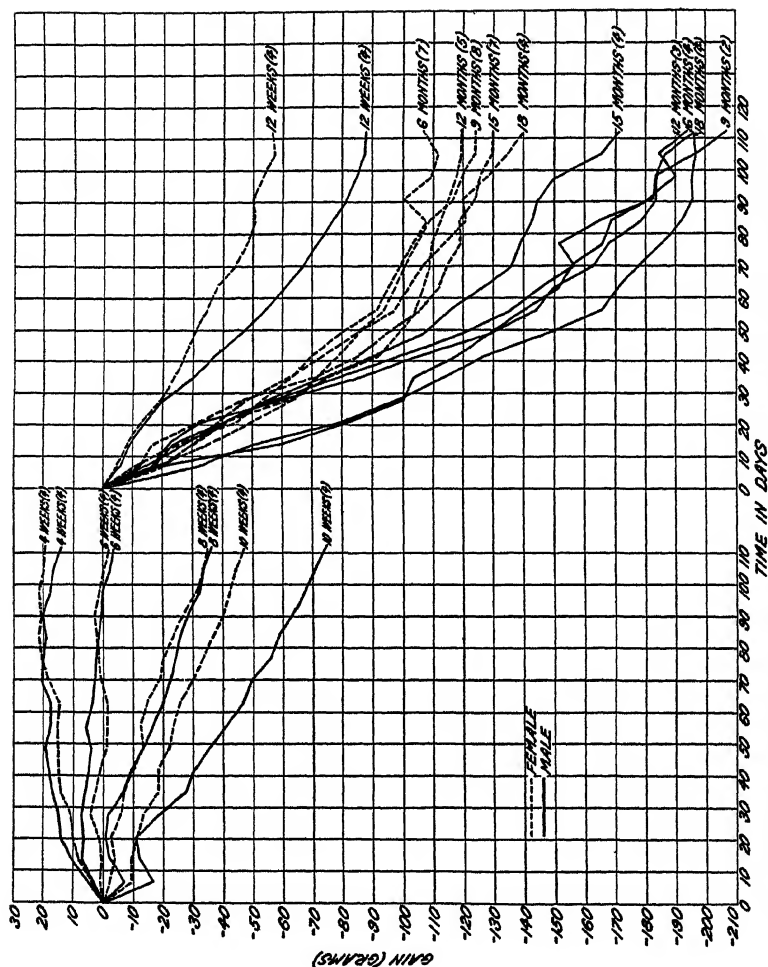


Fig. 4 Weight changes made by male and female rats of different ages on a diet deficient in vitamin G. The number of animals in the group and their age at the beginning of the test are indicated at the end of each respective growth curve.

TABLE 2

Changes in weight expressed as percentage of the initial weight of male and female rats between 4 weeks and 18 months of age on a diet deficient in vitamin G

AGE AT BEGINNING OF TEST	SEX	WEEKS ON EXPERIMENTAL DIET															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
4 weeks	F	Per cent 8.1	Per cent 15.4	Per cent 18.1	Per cent 19.9	Per cent 25.7	Per cent 27.1	Per cent 27.1	Per cent 27.5	Per cent 26.6	Per cent 32.5	Per cent 36.5	Per cent 38.3	Per cent 36.5	Per cent 38.0	Per cent 35.6	Per cent 34.4
	M	8.8	18.5	24.5	25.3	28.7	30.4	32.9	29.5	29.5	34.6	33.7	32.0	34.1	29.8	27.8	22.8
6 weeks	F	1.2	0.6	1.9	5.3	3.7	1.2	- 1.5	- 1.9	- 1.9	0.4	1.9	1.6	3.1	1.2	- 0.9	- 3.1
	M	4.2	8.2	7.0	8.2	6.7	5.6	4.8	6.5	4.2	3.1	2.6	1.1	0.3	0	- 3.0	- 4.5
8 weeks	F	-1.7	-2.8	- 2.1	- 4.6	- 5.7	- 8.3	-11.1	-10.3	-12.4	-15.7	-17.0	-19.6	-22.8	-26.1	-27.8	-29.5
	M	-4.7	- 1.6	- 0.6	- 1.0	- 4.5	- 6.8	- 9.3	-11.9	-14.0	-15.7	-17.0	-18.1	-20.1	-22.5	-23.2	-25.7
10 weeks	F	-7.5	- 7.3	- 8.9	-10.7	-14.7	-14.7	-17.5	-18.7	-20.4	-23.8	-26.3	-28.7	-32.1	-33.5	-35.5	-37.5
	M	-8.3	- 5.9	- 5.4	- 9.1	-13.7	-15.0	-17.6	-20.6	-23.1	-24.3	-27.4	-28.8	-31.4	-33.2	-34.8	-36.5
12 weeks	F	-3.4	- 5.8	- 8.9	-13.6	-16.6	-19.0	-21.0	-23.7	-25.9	-30.0	-33.0	-34.6	-34.4	-36.4	-39.0	-38.3
	M	-2.7	- 4.2	- 6.9	- 9.6	-14.4	-17.6	-21.6	-25.2	-28.4	-31.2	-33.5	-35.7	-38.0	-39.6	-40.6	-41.0
6 months	F	-5.1	-11.9	-20.7	-24.7	-29.3	-32.2	-36.4	-41.8	-43.4	-45.3	-47.7	-49.6	-46.0	-50.2	-51.5	-49.3
	M	-8.9	-14.2	-22.1	-27.3	-31.7	-35.6	-41.2	-46.2	-47.6	-49.5	-51.6	-53.5	-54.4	-54.4	-54.8	-54.7
9 months	F	-8.9	-13.0	-16.7	-21.0	-27.2	-31.8	-36.6	-40.4	-42.0	-43.5	-45.0	-46.7	-50.3	-51.4	-53.3	-53.5
	M	-5.1	- 6.9	-11.8	-16.7	-22.8	-28.9	-34.5	-39.0	-41.8	-45.2	-43.7	-47.5	-52.7	-53.0	-56.7	-59.6
12 months	F	-8.0	-12.6	-16.8	-25.8	-31.5	-37.7	-40.2	-42.5	-43.6	-44.5	-44.8	-46.2	-47.1	-48.2	-48.8	-48.9
	M	-4.4	- 5.8	- 8.8	-15.7	-22.3	-28.1	-33.8	-36.2	-39.3	-40.5	-43.2	-43.9	-47.2	-49.4	-48.0	-50.5
15 months	F	-7.1	-14.0	-19.5	-24.9	-28.1	-33.4	-37.2	-41.2	-43.7	-44.9	-46.7	-46.6	-48.4	-49.0	-50.8	-50.8
	M	-4.4	- 8.3	-12.6	-17.0	-22.6	-27.5	-32.6	-34.9	-38.5	-41.6	-42.3	-43.5	-44.1	-46.1	-50.5	-52.3
18 months	F	-2.8	- 6.2	-12.0	-17.5	-26.4	-29.3	-31.3	-35.9	-37.8	-39.4	-42.1	-44.9	-44.6	-47.6	-49.9	-51.9
	M	-4.9	-15.4	-21.4	-26.0	-26.9	-30.6	-33.5	-37.3	-38.9	-42.2	-43.7	-46.3	-47.7	-47.7	-48.5	-51.1

different age groups made the same response since it has been shown earlier in this paper that the older the animal up to 6 months, the greater was the percentage weight loss suffered.

The data in figure 1 show no evidence of a difference in the survival of the two sexes on a diet low in vitamin G.

Contrary to the findings of Leader ('30) and Thatcher ('31) our animals showed no differences in behavior with the seasons. An explanation for the seasonal variation in the incidence of pellagra in human beings noted by Goldberger ('20) may be readily explained by the change in diet of these people during the year. Following the months in which fresh fruits and vegetables are less plentiful, severe outbreaks of the disease occur. However, in the case of the experimental animals whose diet and living conditions are practically constant at all times, it does not seem plausible to expect a seasonal change in their behavior.

CONCLUSIONS

Groups of rats ranging in age from 1 to 18 months inclusive were placed on a diet deficient in vitamin G. Data show that:

1. Animals 4 weeks old at the beginning of the test gained about 20 gm. during the first 3 months and thereafter maintained approximately this weight for the remainder of the year. Rats 6 weeks old maintained their initial weight for more than a year with little variation. Rats 8, 10, and 12 weeks, and 6, 9, 12, 15, and 18 months lost weight from the start. The older the animal up to 6 months of age, the more rapid was the loss. Age groups between 6 and 18 months showed very similar weight losses. After reaching a certain low weight level characteristic for each group, the animals maintained an approximately constant weight throughout the test.

2. Up to 6 months of age the older the animal the greater was the percentage of its initial weight lost. Greater differences in percentage losses occurred between animals 4, 6, and 8 weeks old than between those 8, 10, and 12 weeks of age.

The older groups showed the same percentage losses in weight.

3. By the end of the twelfth week of the test, the male rats in the various age groups were 157 to 218 gm. below the weight of normal animals of the same age and sex.

4. Groups of rats with initial ages of 4 to 12 weeks suffered only one loss from each group by the twentieth week while most of the animals in groups 6 to 18 months were dead. While the younger animals survived longer than the older ones, both died long before their normal life span had been covered.

5. Except for a tendency to more marked alopecia in the younger animals, the various age groups showed no difference upon autopsy in the type or severity of the gross symptoms of vitamin G deficiency.

6. Male and female rats of the same age, between 4 weeks and 18 months, made very comparable responses when these are considered as percentage of the initial weight gained or lost. The male rats, possessing greater initial weights, showed greater actual weight losses. In the younger animals there was a tendency for the males to make slightly greater responses than the females followed by a more rapid decline. The survival behavior of the two sexes appears to be the same for a given age.

7. There was no seasonal variation noted in the development of symptoms of vitamin G deficiency.

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STUDIES IN HUMAN PHYSIOLOGY

V. URINE CHEMISTRY; COMPARISON OF 24-HOUR AND SHORT-PERIOD, BASAL EXCRETION; CORRELATIONS BETWEEN URINE CONSTITUENTS AND MENSTRUAL AND SEASONAL VARIATION

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TWO FIGURES

(Received for publication April 20, 1933)

In the preceding reports of this series (Griffith et al., '29) will be found a record of the intra-individual variations of body temperature and basal metabolism, pulse rate and blood pressure, alveolar air, blood gas capacity and respiration of five normal adults who were under observation during the 2-year period, February, 1925, through January, 1927. Two of the subjects, A.B. and C.D., were men and served continuously throughout the entire period; the remaining three subjects were women and of them, two, E.F. and G.H., were under observation during the first year (February, 1925, through January, 1926), while the third, K.L., took their place during the second year (February, 1926, through January, 1927). For the first year, which will hereafter be referred to as '1925' (February, 1925, through January, 1926) there are, therefore, contemporaneous data for two men (A.B. and C.D.) and two women (E.F. and G.H.); during the succeeding year, '1926' (February, 1926, through January, 1927) the group was composed of the same two men and the third woman, K.L.; the first paper of this series should be consulted for further details in regard to the age, weight, and occupation.

It is the purpose of this report to present a summary of the data derived from analysis of the urine of these subjects.

METHOD

The primary purpose of this study was to determine the extent of variability that might be expected under normal conditions. To that end no observations are reported here that were made when there was any doubt as to the physical fitness of the subjects. Nor were there any restrictions as to diet, except one; viz., no meat was eaten during the day of the collection of the 24-hour urines during the first year, nor, as will be explained later, during the day preceding the collection of the short-period, morning urines of the second year; otherwise the subjects ate according to their desire both as to amount and kind of food. This single restriction was imposed in order to eliminate the possibility of an unusual stimulation of the basal metabolic rate, which was one of the chief matters under investigation, through the specific dynamic action of an occasional, excessive protein ingestion. The results would seem to indicate that protein intake was not unduly lowered, being kept normal, but, it is hoped, never becoming excessive, through the meat substitutes (eggs, milk, etc.) employed.

The data for 1925 are derived from 24-hour urines that were collected approximately once a week throughout the year during a day of no meat ingestion, as mentioned above.

Following this day during which no meat was eaten, the subjects came to the laboratory, the first thing in the morning, for determination of the basal metabolism and other measurements as previously reported. During 1926, these determinations were made approximately twice a week on each of the three subjects then serving. And during this year, instead of 24-hour urines, samples were obtained that corresponded with the period of the metabolism determinations; the bladder was emptied, noting the time, just before lying down; the urine was again passed and saved, and the time noted, upon getting up. The preliminary rest period preceding the

metabolism determinations was about 1 hour; the determination of the metabolic rate and the other measurements which were made in connection with it occupied approximately another hour (see the last item of table 1); so these (1926) samples represent the urine secretion under basal conditions early in the morning and after a day of controlled protein intake.

Since A.B. and C.D. served through both years without change in habit or diet, their data are particularly instructive for the comparison which they afford between 24-hour excretion and that occurring under basal conditions.

The methods of analysis were all standard and descriptions of them may be found in the following citations from Hawk and Bergeim's *Practical Physiological Chemistry*, ninth edition:

Urea: Aeration method of Cullen and Van Slyke, page 720.

Ammonia: Permutit method of Folin and Bell, page 727.

Creatinine: Folin's colorimetric method, page 733.

Creatin: Folin's method, page 735.

Uric acid: Colorimetric method of Benedict and Frank, page 736.

Amino acids: Colorimetric method of Folin, page 731.

Total nitrogen: Method of Folin and Wright, page 713.

Organic acids: (Corrected for creatinine and amino acids) Van Slyke and Palmer, page 706.

Total acid: Folin's method, page 705.

Sugar: Folin and Berglund, page 753.

Chlorides: Whitehorn, page 401.

Phosphorus: Fiske and Subbarow, page 776.

All analyses were done with standardized apparatus in duplicate and repeated when necessary until the customary checks were obtained.

I. STATISTICS

1. *Averages*; see table 1. In using the table it should be noticed that the data from the short-period urines of 1926 have been computed to a 24-hour basis in order to facilitate comparison with the 24-hour samples of 1925.

TABLE 1

Statistics of urinary excretion. The urines of 1925 were 24-hour, and those of 1926 short-period, morning, basal samples (the last entry of the table gives the average times of collection). For purposes of comparison all values are in terms of excretion per 24 hours

FUNCTION	SUBJECT	YEAR	NUMBER OF DETERMINATIONS	CUBIC CENTIMETERS AND GRAMS PER 24 HOURS			STANDARD DEVIATION	COEFFICIENT OF VARIATION
				Minimum and maximum	Mode	Arithmetical mean		
Volume cubic centimeters per 24 hours	A.B.	1925	52	550-1850	1000	1000±25	272.0	24.7
		1926	86	600-4800	1920; 2280; 2640	2554±60	829.6	32.5
	C.D.	1925	51	650-2000	2880; 3240			
		1926	85	480-2400	1050; 1150	1086±23	242.0	22.3
	E.F.	1925	48	400-2100	960	1080±29	394.8	36.6
		1926	86	400-2100	650	950±32	325.0	34.2
	G.H.	1925	46	350-1200	600	663±16	159.5	24.1
		1926	81	240-5160	1920; 3000	2344±98	1308.0	56.0
Specific gravity	A.B.	1925	50	1.005-1.038	1.021	1.0219±0.0004	0.006	0.54
		1926	86	1.002-1.026	1.010; 1.011	1.0115±0.0003	0.004	0.43
	C.D.	1925	51	1.012-1.038	1.025	1.0243±0.0005	0.005	0.50
		1926	82	1.008-1.036	1.024	1.0116±0.0004	0.005	0.49
	E.F.	1925	48	1.011-1.031	1.018	1.0212±0.0006	0.006	0.57
		1926	45	1.012-1.039	1.027	1.0259±0.0007	0.007	0.69
	K.L.	1925	78	1.002-1.039	1.008; 1.010	1.0135±0.0006	0.010	0.94
		1926	81	1.002-1.039	1.008; 1.010	1.0135±0.0006	0.010	0.94
Urea N grams per 24 hours	A.B.	1925	52	5.60-11.00	7.4; 7.8	7.760±0.110	1.13	14.5
		1926	86	5.28-11.76	8.52	8.200±0.090	1.23	15.0
	C.D.	1925	49	5.20-13.60	8.80	8.580±0.150	1.52	17.8
		1926	85	5.52-12.24	8.40	7.870±0.090	1.19	16.1
	E.F.	1925	48	3.00- 7.20	5.00	5.442±0.094	0.97	17.8
		1926	46	2.60- 9.00	5.60	5.448±0.144	1.45	26.6
	K.L.	1925	81	1.92-11.28	7.20	7.090±0.126	1.68	23.7
		1926	81	1.92-11.28	7.20	7.090±0.126	1.68	23.7
Ammonia-N gram per 24 hours	A.B.	1925	52	0.20-0.61	0.43	0.397±0.009	0.091	23.0
		1926	86	0.12-0.65	0.26	0.285±0.006	0.086	30.3
	C.D.	1925	51	0.40-0.84	0.48	0.560±0.010	0.105	18.7
		1926	85	0.10-0.96	0.38	0.469±0.013	0.177	37.7
	E.F.	1925	48	0.24-0.74	0.46	0.483±0.010	0.104	21.5
		1926	44	0.12-0.84	0.24	0.282±0.015	0.144	51.1
	K.L.	1925	81	0.00-0.50	0.22	0.214±0.007	0.093	43.4
		1926	81	0.00-0.50	0.22	0.214±0.007	0.093	43.4
Creatinine-N grams per 24 hours	A.B.	1925	52	0.20-0.62	0.43; 0.44	0.442±0.005	0.053	12.7
		1926	83	0.31-0.50	0.41	0.415±0.002	0.033	8.1
	C.D.	1925	51	0.43-0.80	0.54; 0.56	0.580±0.007	0.075	12.9
		1926	85	0.41-0.73	0.61	0.600±0.004	0.051	8.4
	E.F.	1925	48	0.27-0.52	0.36; 0.38; 0.39	0.384±0.004	0.045	11.8
		1926	46	0.15-0.44	0.42	0.323±0.008	0.078	24.2
	K.L.	1925	79	0.12-0.43	0.29; 0.37; 0.44	0.358±0.003	0.043	12.2
		1926	79	0.12-0.43	0.35	0.358±0.003	0.043	12.2
Creatine-N grams per 24 hours	A.B.	1925	51	0-0.092	0.022; 0.030	0.041±0.002	0.022	53.5
		1926	76	0-0.050	0	0.012±0.001	0.013	104.0
	C.D.	1925	51	0-0.120	0.035	0.049±0.003	0.029	60.0
		1926	80	0-0.086	0	0.027±0.002	0.023	86.2
	E.F.	1925	47	0.004-0.086	0.048	0.047±0.002	0.021	44.2
		1926	45	0-0.180	0.050	0.045±0.004	0.035	78.0
	K.L.	1925	75	0-0.089	0	0.022±0.002	0.019	86.8
		1926	75	0-0.089	0	0.022±0.002	0.019	86.8
Uric acid-N grams per 24 hours	A.B.	1925	52	0.070-0.260	0.150	0.162±0.004	0.043	26.3
		1926	84	0.084-0.372	0.180	0.188±0.003	0.037	19.8
	C.D.	1925	51	0.110-0.330	0.150	0.185±0.004	0.047	25.6
		1926	85	0.108-0.528	0.228	0.220±0.004	0.054	24.6
	E.F.	1925	48	0.080-0.250	0.160	0.158±0.004	0.039	24.7
		1926	46	0.080-0.240	0.120	0.127±0.004	0.045	35.6
	K.L.	1925	78	0.086-0.204	0.156	0.153±0.003	0.033	21.6
		1926	78	0.086-0.204	0.156	0.153±0.003	0.033	21.6

TABLE 1—Continued

FUNCTION	SUBJECT	YEAR	NUMBER OF DETERMINATIONS	CUBIC CENTIMETERS AND GRAMS PER 24 HOURS			STANDARD DEVIATION	COEFFICIENT OF VARIATION
				Minimum and maximum	Mode	Arithmetical mean		
Amino acid-N grams per 24 hours	A.B.	1925	51	0.050-0.164	0.082	0.098±0.002	0.023	23.7
		1926	82	0-0.149	0.082; 0.094 0.108; 0.132	0.098±0.002	0.027	27.5
	C.D.	1925	51	0.085-0.185	0.100	0.120±0.002	0.025	20.9
		1926	85	0.066-0.174	0.108	0.110±0.002	0.030	27.1
	E.F.	1925	48	0.060-0.290	0.100	0.114±0.004	0.042	37.1
	G.H.	1925	46	0.040-0.200	0.060; 0.080	0.090±0.004	0.036	39.4
	K.L.	1926	75	0.036-0.156	0.096	0.098±0.000	0.021	21.5
Total nitrogen grams per 24 hours	A.B.	1925	51	7.0-12.6	9.6; 10.0	9.40±0.11	1.17	12.5
		1926	84	7.0-13.0	8.8; 9.2; 9.5; 9.8	9.30±0.09	1.21	13.0
	C.D.	1925	51	7.4-16.4	11.6	10.63±0.16	1.73	16.3
		1926	85	6.7-15.1	10.3	9.83±0.10	1.39	14.1
	E.F.	1925	48	4.8- 9.2	6.6	7.09±0.11	1.11	15.6
	G.H.	1925	46	3.2-10.4	6.6	6.85±0.17	1.68	24.7
	K.L.	1926	81	2.4-12.7	9.6	8.35±0.13	1.74	20.8
Rest nitrogen grams per 24 hours	A.B.	1925	51	0.050-1.350	0.350	0.462±0.025	0.268	58.0
		1926	75	0.024-0.984	0.288; 0.300	0.450±0.022	0.286	63.6
	C.D.	1925	49	0.200-1.150	0.550	0.600±0.020	0.212	35.3
		1926	84	0.072-1.512	0.624	0.583±0.018	0.239	41.0
	E.F.	1925	47	0.100-1.500	0.350	0.472±0.027	0.270	57.2
	G.H.	1925	45	0.050-1.300	0.300	0.449±0.029	0.285	63.5
	K.L.	1926	70	0.048-0.840	0.552	0.393±0.016	0.194	49.4
Organic acids cubic centimeters 0.1 N acid per 24 hours. Corrected for creatinine and amino acids	A.B.	1925	51	330-670	460	505.7± 7.8	82.5	16.3
		1926	73	0-756	420	468.8±12.5	158.4	33.8
	C.D.	1925	47	390-930	540; 570; 600; 610; 660	614.5±11.1	113.0	18.4
		1926	71	0-792	528	528.0±10.9	136.3	25.8
	E.F.	1925	45	350-930	550	569.3±11.5	115.0	20.2
	G.H.	1925	42	180-920	520	432.4±15.5	149.0	34.5
	K.L.	1926	62	276-744	408	493.5± 9.8	114.9	23.3
Sugar grams per 24 hours	A.B.	1925	52	0.280-1.000	0.520; 0.620	0.552±0.012	0.128	23.2
		1926	84	0.252-0.516	0.312; 0.336	0.361±0.004	0.058	16.0
	C.D.	1925	51	0.300-0.900	0.620	0.718±0.011	0.118	16.4
		1926	85	0.192-0.264	0.384	0.404±0.005	0.063	15.6
	E.F.	1925	47	0.200-0.820	0.560	0.479±0.012	0.120	25.1
	G.H.	1925	46	0.200-1.500	0.500	0.531±0.023	0.226	42.6
	K.L.	1926	78	0.108-0.528	0.396	0.352±0.006	0.075	21.4
Phosphate grams per 24 hours	A.B.	1926	84	0.156-0.756	0.372	0.371±0.008	0.113	30.5
	C.D.	1926	85	0.216-1.056	0.648	0.588±0.012	0.117	28.4
	K.L.	1926	76	0.216-1.200	0.552; 0.576	0.596±0.014	0.174	29.2
Chloride grams per 24 hours	A.B.	1926	82	5.4-24.6	12.0	15.024±0.337	4.52	30.1
	C.D.	1926	83	3.6-19.8	8.4; 9.6	9.558±0.244	3.30	34.5
	K.L.	1926	76	3.6-21.0	10.2	9.710±0.272	3.52	36.3
Total acid cubic centimeters 0.1 N acid per 24 hours	A.B.	1926	72	Alk.-288	36	70.0±4.4	55.7	79.5
	C.D.	1926	76	Alk.-456	84	163.6±4.0	51.1	31.2
	K.L.	1926	72	Alk.-504	84	153.6±8.3	103.9	19.3
Time of collection, 1926 Minutes	A.B.		86	90-186	134	135		
	C.D.		85	92-238	164	165		
	K.L.		81	94-180	120	134		

Aside from establishing norms for the intra-individual variation in excretion to be expected over a period of time, perhaps one of the most interesting uses of the table is the comparison which it affords between the rates of excretion under basal conditions in the morning (1926) and the average 24-hour rate (1925). This comparison can be strictly made only with the data of the two men, A.B. and C.D., since they only furnished both 24-hour and short-period urines; and, even with them, the comparison is subject to the limitation that the urines to be compared were secured a year apart. As mentioned before, however, it is believed that there were no differences in diet or habits during the 2 years sufficient to invalidate the comparison and that the averages for each year are based on a sufficient number of observations to exclude any gross errors due to limitation of sampling.

Assuming, then, that the data for A.B. and C.D. furnish an approximately valid comparison between the average rate of excretion during 24-hour periods and that under post-absorptive, basal conditions for short periods in the morning, it is somewhat surprising to find how little consistent difference they show. Ammonia, creatine and sugar are the only substances that have a definitely lowered rate of excretion in the basal morning urines. The maxima and minima, the modes and averages are either quite alike or fail to show consistent differences in the two subjects for the other data, with the exception of uric acid which shows a higher rate of excretion under basal conditions for both.

The volume of the urine under the two conditions is worthy of remark because of the exceptional behavior of C.D. With A.B., the rate of excretion of the short-period urines is, as an average, two and one-half times greater than the average 24-hour rate; this was to be expected both from the effect of recumbency (White, Rosen, Fischer and Wood, '26) and the fact that they were obtained in the morning (Simpson, '24). A comparison of the data for K.L. with that for the other two women subjects, E.F. and G.H., though by no means a very valid one, would seem, also, to substantiate the recumbent and matutinal effects on volume. With C.D., however, the

average 24-hour rate is almost exactly that computed from the short-period samples obtained during the morning while lying down.

This difference in the behavior of the volume may perhaps be of significance in explaining the findings in regard to urea, since, as is well known and will be more fully developed in a following section, the two are to a certain degree correlated. The rate of urea excretion of A.B. is less in the 24-hour samples than in those obtained under basal conditions in which the protein metabolism must have been minimal and predominantly endogenous; and a similar difference is again indicated, though less validly, by comparison of the rates of excretion of the three women subjects, E.F., G.H. (1925, 24-hour urines), and K.L. (1926, basal, morning urines). On the other hand, C.D., who fails to show the matutinal and recumbent augmentation of volume has a lower rate of excretion for urea under basal conditions (1926) than in the 24-hour samples (1925) where the amount is no doubt increased from exogenous sources.

The greater rate of creatine excretion in the 24-hour urines than under basal conditions may be taken as evidence in support of at least partly exogenous origin for the amount of this substance normally excreted (Hunter, '22, p. 610); and the same may be said for sugar (Folin and Berglund, '22 a). In addition, attention may be called to the almost identical average excretion of creatin by the men and women of this group; there is no evidence of a sex difference, except that the men are more variable, as will be shown in a following section.

The average 24-hour output of ammonia is no doubt raised by the high level of excretion during sleep which many have reported (Simpson, '24, '26); whereas the morning urines may have partaken of the alkaline tide following awakening (Hubbard, '30) with concomitant retention of this substance (Bazett, '24).

There is previous evidence to prepare us for the greater rate of uric acid excretion in the basal, morning urines than in the 24-hour samples; its cause is uncertain. It can hardly

be due to the matutinal diuresis, for, although it has been claimed (Goiffon, '28) that the concentration of uric acid tends to remain fixed, whatever the output of water, our own data do not substantiate this. In the first place the correlation between uric acid and volume, especially in the short-period urines, is too low to be significant (see below); and, secondly, the increased output in the morning urines is even more pronounced in the subject C.D. than with A.B., although the former showed no morning diuresis. Lennox ('25) has shown that the blood uric acid rises during a fast, but he was inclined to attribute this to retention, which would decrease rather than increase the urinary output; moreover, Hubbard ('27) has shown that within the time after the last meal represented by our morning urines (16 to 18 hours) this increase in blood concentration has not become noticeable. Finally, there is nothing except individual preference to guide one in choosing between the suggestion by Smetanka ('12, '13) of a delayed effect of the previous meal; and that of Cathcart, Kennaway and Leathes ('07) of increased muscular activity, if only of tonus, on awakening.

Creatinine and amino acids both show remarkably similar rates of excretion in the 24-hour and short-period, morning urines. This was not unexpected of the former and may be taken as confirmation of the views of Folin and of Shaffer (Hunter, '22, p. 593) regarding the constant metabolism of this substance. It may be mentioned as a matter of record that the creatinine coefficients of these subjects fall within expected ranges, being, for the men, 8.1 (A.B.) and 9.8 (C.D.) with an average of 8.9; and for the women, 7.2 (E.F.), 5.8 (G.H.), and 6.4 (K.L.), with an average of 6.5.

On the other hand so little is known in regard to amino acid metabolism that it is difficult to appraise the significance of the similar rates of excretion of this fraction in the 24-hour and short-period, morning urines. From the work of Folin and Berglund ('22 b), as well as other work to which they refer, it might have been expected that the 24-hour excretion would be augmented by the greater output occurring after meals, especially since there seems to be no threshold for these sub-

stances, and it is claimed for them (*loc. cit.*, p. 411) that the rate of excretion is independent of diuresis. This last, however, may need revision and it is then not impossible that the conditions here are similar to those affecting urea as described above. This is suggested by the relatively high correlation (see below) between amino acids and urea and amino acids and volume (especially in the short-period samples) as well as the fact that C.D., who, as explained above, failed to show the matutinal diuresis, also has a slightly lower average rate of amino acid excretion in the morning urines than in the 24-hour samples.

2. *Variability.* The following tabulation affords a comparison, in terms of the coefficient of variation, of the variability of excretion of these substances in the 24-hour and short-period, basal morning urines. The figures are the averages for all subjects serving in each year, derived from table 1:

<i>Coefficients of variation</i>	
<i>1925</i> 24-hour urines	<i>1926</i> Short-period urines
Specific gravity 0.6	0.6.....Specific gravity
Creatinine15.4	9.6.....Creatinine
Total nitrogen17.3	15.9.....Total nitrogen
Urea19.2	17.6.....Sugar
Organic acid22.3	18.3.....Urea
Volume26.3	22.0.....Uric acid
Sugar26.8	25.4.....Amino acid
Uric acid28.1	27.6.....Organic acid
Ammonia28.6	29.4.....Phosphates
Amino acids29.8	33.6.....Chlorides
Rest nitrogen53.5	37.1.....Ammonia
Creatine58.9	41.7.....Volume
	43.3.....Total acid
	51.3.....Rest nitrogen
	92.3.....Creatine

These figures show that there is little significant difference either in the absolute or relative variability of the majority of these substances in the two types of urines. The only appreciable exceptions are the greater variability of sugar in the 24-hour urines and of volume (per unit time in both) in the short-period samples. It may be noted that creatine, the most variable constituent of both urines, is much more variable in the short-period, basal ones.

In the last of the previous numbers of this series (Griffith et al., '29 d, p. 569) may be found a table of the coefficients of variation of the physiological functions previously reported upon; these were twenty-nine in number and of them eighteen, or practically two-thirds, were more variable in the women than in the men. Attention may be recalled to the warning already given (Griffith et al., '29 c, p. 454; '29 d, p. 567) against uncritical generalization from such limited data as we have; nevertheless, merely as a description of our results, it is interesting to note that this apparent sex difference continues to hold in even greater proportion for the data presented here. This may be seen from the following tabulation compiled from the data of table 1:

Average coefficients of variation for the men and women of our group and grand averages for both sexes and both types of urine

	Men	Women	Grand average
¹ Specific gravity,	0.5	0.7	0.6
¹ Creatinine,	10.5	16.1	12.9
¹ Total nitrogen,	14.0	20.4	16.6
¹ Urea,	15.9	22.7	18.8
¹ Sugar,	17.8	29.7	22.9
¹ Organic acids,	23.6	26.0	24.6
¹ Uric acid,	24.1	27.3	25.5
¹ Amino acids,	24.8	32.7	28.0
Phosphates,	29.4	29.2	29.4
¹ Ammonia,	27.4	38.7	32.2
¹ Volume,	29.0	38.1	32.9
¹ Chlorides,	32.3	36.3	33.6
Total acid,	55.3	19.3	43.3
¹ Rest nitrogen,	49.5	55.7	52.6
Creatine,	75.9	67.3	73.2

¹ Women more variable than the men.

Thus, with the exception of phosphates, total acid and creatine, the average variability of the women is greater than that of the men. As was mentioned in the previous section, this greater variability of creatine excretion in the men than in the women is the only evidence these data afford in support of the widely held belief that creatinuria in the adult is essentially a female prerogative (see Hunter, '22, p. 612). As far as the actual, average amounts excreted over a period of time are concerned, the two sexes are quite alike, as may be seen from table 1.

Referring again to the table in the preceding report, it may be seen that for twenty-seven of the twenty-nine functions recorded there, the coefficients of variation range from 0.5 to 12.9. The remaining two, with coefficients greater than this (18.3), are for the protein oxygen and carbon dioxide, derivable from the data of this paper. It would appear, therefore, that the variability in excretion of the urinary constituents stands relatively high as compared with the metabolic, respiratory and cardio-vascular functions previously reported; for, of these urinary data, only the specific gravity and creatinine under basal conditions have coefficients of variation less than 12.9. That our coefficients are not abnormally high would seem to be apparent from a comparison with the only other data of similar kind, those of C. P. White, who gives the following figures, alongside which are placed our corresponding figures in parenthesis:

Urea,	30.7	(18.9)
Phosphates,	37.2	(29.4)
Chlorides,	44.3	(33.6)
Uric acid,	45.1	(25.5)
Ammonia,	50.0	(32.2)

3. *Correlations.* Following are given the coefficients of correlation for simultaneous variations in rate of excretion of the urinary constituents for which we have record. The left-hand entries are for 24-hour, and the right-hand for the short-period, basal, morning urines.

1. Correlations with volume

	+0.63.....	Volume, chlorides
	0.45.....	Volume, amino acids
	0.33.....	Volume, urea
Volume, creatinine	0.31.....	Volume, sugar
	0.29.....	Volume, creatine
Volume, urea	0.25	
Volume, uric acid	0.23	
Volume, amino acids	0.22	
Volume, organic acids	0.21	
	0.17.....	Volume, organic acids
	0.16.....	Volume, uric acid
	0.12.....	Volume, creatinine
Volume, creatine	0.08	
Volume, sugar	-0.01	
	0.02.....	Volume, ammonia
	0.08.....	Volume, phosphates
Volume, ammonia	0.13	

2. Correlations with urea

Urea, ammonia	+0.49	
Urea, uric acid	0.40	
	0.37.....	Urea, amino acid
Urea, creatinine	0.36.....	Urea, phosphate
	0.35.....	Urea, creatinine
	0.33.....	Urea, volume
	0.32.....	Urea, sugar
Urea, amino acids	0.31	
	0.30.....	Urea, uric acid
Urea, volume	0.25.....	Urea, chloride
	0.23.....	Urea, ammonia
		Urea, organic acid
Urea, organic acid	0.19	
	0.17.....	Urea, creatine
Urea, sugar	0.12	
Urea, creatine	0.01	

3. Correlations with ammonia

Ammonia, urea	+0.49	
	0.23.....	Ammonia, urea
	0.21.....	Ammonia, phosphate
Ammonia, sugar	0.16	
Ammonia, organic acids	0.15	
	0.14.....	Ammonia, creatinine
Ammonia, amino acids	0.10	
	0.07.....	Ammonia, sugar
Ammonia, creatinine	0.06	
	0.05.....	Ammonia, amino acids

Ammonia, creatine	0.02.....	Ammonia, creatine
Ammonia, uric acid	Ammonia, uric acid
	-0.02.....	Ammonia, chlorides
	0.11.....	Ammonia, volume
Ammonia, volume	0.13.....	Ammonia, organic acids

4. Correlataions with creatinine

Creatinine, uric acid	+0.54	
Creatinine, urea	0.36	
	0.35.....	Creatinine, urea
Creatinine, volume	0.31	
Creatinine, sugar		
Creatinine, organic acid	0.28	
	0.27.....	Creatinine, uric acid
	0.22.....	Creatinine, phosphates
	0.21.....	Creatinine, amino acids
Creatinine, amino acids	0.18	
	0.17.....	Creatinine, sugar
	0.14.....	Creatinine, ammonia
	0.12.....	Creatinine, volume
	Creatinine, organic acid
Creatinine, ammonia	0.06	
	-0.04.....	Creatinine, chloride
	0.11.....	Creatinine, creatine
Creatinine, creatine	0.23	

5. Correlations with creatine

	+0.29.....	Creatine, volume
Creatine, organic acid	0.28	
	0.24.....	Creatine, chloride
	0.22.....	Creatine, sugar
	0.17.....	Creatine, urea
Creatine, volume	0.08	
	0.06.....	Creatine, amino acid
	0.03.....	Creatine, phosphate
Creatine, ammonia	0.02.....	Creatine, ammonia
Creatine, urea	0.01	
Creatine, amino acid	-0.01.....	Creatine, uric acid
Creatine, uric acid	0.03	
	0.07.....	Creatine, organic acid
Creatine, sugar	0.11.....	Creatine, creatinine
Creatine, creatinine	0.23	

6. Correlataions with uric acid

Uric acid, creatinine	+0.54	
Uric acid, urea	0.40	
Uric acid, sugar	0.34	
	0.30.....	Uric acid, urea
	0.27.....	Uric acid, creatinine
	0.24.....	Uric acid, amino acid

Uric acid, volume	0.23
Uric acid, organic acid	0.22
Uric acid, amino acid	0.19
	0.17.....Uric acid, phosphates
	0.16.....Uric acid, volume
Uric acid, chlorides
	0.13.....Uric acid, organic acids
	0.12.....Uric acid, sugar
Uric acid, ammonia	0.02.....Uric acid, ammonia
	-0.01.....Uric acid, creatine
Uric acid, creatine	0.03

7. Correlations with amino acids

	+0.45.....Amino acids, volume
	0.37.....Amino acids, urea
Amino acids, sugar
Amino acids, organic acid	0.35
	0.32.....Amino acids, chlorides
Amino acids, urea	0.31
	0.24.....Amino acids, uric acid
Amino acids, volume	0.22
	0.21.....Amino acids, creatinine
	0.20.....Amino acids, organic acid
Amino acids, uric acid	0.19
Amino acids, creatinine	0.18
Amino acids, ammonia	0.10
	0.06.....Amino acids, creatine
	0.05.....Amino acids, ammonia
	0.04.....Amino acids, phosphates
Amino acids, creatine	-0.01
Amino acids, sugar	

8. Correlations with sugar

	+0.37.....Sugar, amino acid
Sugar, uric acid	0.34
	0.32.....Sugar, urea
Sugar, creatinine	0.31.....Sugar, volume
	0.23.....Sugar, chlorides
Sugar, organic acid	0.22.....Sugar, creatine
	0.17.....Sugar, creatinine
Sugar, ammonia	0.16
	0.14.....Sugar, organic acid
Sugar, urea	0.12.....Sugar, uric acid
	0.11.....Sugar, phosphate
	0.07.....Sugar, ammonia
Sugar, volume	-0.01
Sugar, amino acid	
Sugar, creatine	0.11

9. *Correlations with chlorides*

(Data for short-period, morning urines, only)

+0.63.....	Chlorides, volume
0.32.....	Chlorides, amino acid
0.25.....	Chlorides, urea
0.24.....	Chlorides, creatine
0.23.....	Chlorides, sugar
0.19.....	Chlorides, organic acid
0.16.....	Chlorides, uric-acid
0.02.....	Chlorides, ammonia
-0.03.....	Chlorides, phosphates
0.04.....	Chlorides, creatinine

10. *Correlataions with phosphates*

(Data for short-period, morning urines, only)

+0.36.....	Phosphates, urea
0.22.....	Phosphates, creatinine
0.21.....	Phosphates, ammonia
0.17.....	Phosphates, uric acid
0.11.....	Phosphates, sugar
0.09.....	Phosphates, organic acid
0.04.....	Phosphates, amino acid
0.03.....	Phosphates, creatine
-0.03.....	Phosphates, chlorides
0.08.....	Phosphates, volume

Too much is not to be made of the absolute size of these coefficients; those for the 24-hour urines are based on approximately 100 analyses and those for the short-period samples on about 225, so the probable errors range from ± 0.04 to ± 0.07 , depending on the magnitude of the coefficient. The smaller coefficients are given, therefore, for comparative purposes only; in order, in the first place, to be able to compare the conditions that obtain in the 24-hour samples with such as are shown by the short-period ones; and, secondly, to compare our results with those that have been or may be reported by others.

Discussion. There is very little previous work directly bearing on this aspect of excretion. Rich has reported ('28) a correlation between creatinine and phosphorus, in 24-hour urines, of $+0.64$. We do not have a strictly comparable figure, since ours of $+0.22$ was obtained only on short-period

urines. Hubbard and Munford ('22) and Hubbard ('23, '24) have shown by a method of analysis somewhat different from ours, but still of a statistical nature, that ammonia excretion varies as the volume of the urine, provided the reaction is constant; otherwise, as in our work, there is no correlation. But the most comparable precedent is to be found in the exhaustive statistical analysis by C. P. White ('22, '25) of the data from 100 urines (presumably 24-hour), fifty of which were derived from hospital patients with cancer of the breast and fifty from hospital patients suffering from other diseases not affecting kidney function. The latter were used as controls in the effort to establish peculiarities of metabolism associated with cancer. For our purpose they may be taken to represent normal kidney function under hospital standardization of exercise and diet, and as such afford an interesting comparison with the data (24-hour) provided by our normal subjects under ordinary living conditions.

Coefficients of correlation

	<i>24-hour urines</i>	
	<i>White Hospital patients 50 samples</i>	<i>Ourselves Normal subjects 100 samples</i>
Volume, creatinine,	+0.54	+0.31
urea,	0.42	0.25
uric acid,	0.40	0.23
ammonia,	0.19	-0.13
Urea, creatinine,	+0.71	+0.36
uric acid,	0.41	0.40
ammonia,	0.38	0.49
Uric acid, creatinine,	+0.27	+0.54

Although all of our values in the first group are lower than White's, it is interesting that the same relative correlation with volume is evident in both. The absolute differences, here, as in the remaining correlations with urea and uric acid, may not improbably reflect the different physiological conditions represented by them.

It is not necessary or expedient to attempt a discussion of the very extensive literature which might bear in some way

on these results. Much of this can be found summarized in the review of Marshall ('26). Nevertheless, there are a few points that deserve attention.

It can be seen that the correlation between volume and chlorides is so much greater than any other as to be in a class apart, and what we have found for the short-period, morning urines (we have chloride determinations for them only) presumably obtains for 24-hour samples also; for C. P. White, in the work already referred to, gives the coefficient of correlation between volume and chlorides as $+0.76$. These are merely definite numerical statements of a fact known qualitatively long since. Thus, Adolph has stated ('23, p. 448) that as between urea and chloride, the latter is the chief factor in determining urine volume; and Griffith and Hansell ('25), Simpson ('24), Marshall and Crane ('22) and White, Rosen, Fischer and Wood ('26) have shown that in the diureses due to abdominal pressure, morning collection, section of splanchnic nerve and recumbency, chloride excretion is preeminently increased (the only exception is the diuresis due to ingestion of water or salt solution (Adolph, '21; H. L. White, '27) in which chloride excretion seems to be independent of volume, or, according to Marshall ('20), less augmented than the output of urea). But even where volume and chloride are related a correlation coefficient is of no value in determining causality. From this point of view, 24-hour urines probably present too complicated a tangle to be at present unraveled. Whether, under the simpler conditions represented by our basal, morning urines, water swept out chloride or the latter determined the volume, would seem to be decided in favor of the latter; for Simpson has shown ('26) that on waking from sleep in the morning the amount of chloride excreted markedly increases whether the volume does so or not; and this primary excess of chloride appears to arise from deep-seated physiological adjustments, particularly of respiration, that occur on transition from sleep to wakefulness (Simpson and Wells, '28).

Marshall and Crane, and H. L. White and his co-workers agree in placing urea and phosphates in an intermediate class whose members, though affected by volume, are not so closely related to it as chloride. This is confirmed by our figures for urea, which show a significant correlation with volume but one approximately only half as great as that between volume and chloride. But, from our data, which are confirmed by the observations of Bock and Iversen ('21, '22), phosphates, with a volume correlation of -0.08 , fall rather in the third class recognized by these investigators (Marshall, White) of substances not affected by volume. In this class they also place creatinine and ammonia. The latter is certainly independent of volume according to our data; and although creatinine shows a fairly high correlation with volume ($+0.31$) in our 24-hour urines, in the short-period samples, which more nearly correspond with those studied by Marshall and by White, its independence of volume ($+0.12$) is apparent. It is not necessary to discuss here the significance of these findings for rival theories of kidney function; this confirmation (on the whole) by an independent statistical method of previous experimental findings gives added interest, however, to the discussions which may be found in the papers referred to (see also Marshall and Crane, '24).

Turning to the correlations among the organic constituents of the urine for evidence of metabolic inter-relationships, three substances, ammonia (excepting its correlation with urea), creatine and sugar (reducing substances) may be dismissed with little comment, as having coefficients either so small or so unlike in the two kinds of urines as to indicate almost complete independence in their origin and excretion. Exceptions to this which may be worth noting are the fairly high correlation between ammonia and urea, just mentioned, and the inverse relationship between creatine and creatinine, which, though low, is similar in both the 24-hour and short-period urines. Too little is known of creatine and creatinine metabolism to do more with this latter fact than record it as a statistical constant.

Correlation between ammonia and urea (or, total nitrogen; notice that the correlations between ammonia and the other nitrogenous components of the urine, amino acids, uric acid, creatinine, are insignificant) has long been known. Hasselbalch ('12, '16) and later Raffin ('26) have claimed that there is a constant ratio between the two at a given pH. Hubbard and Munford ('22) and later Hubbard have revised this to the extent of showing that the highest correlation is between urinary acidity and ammonia concentration, rather than total output—a fact which is taken to favor the renal origin of ammonia for neutrality regulation (Nash and Benedict, '21; Benedict and Nash, '29) as against its formation in the tissues, generally (Bliss, '26; Artom, '26). More recently, however, Polonovski and Boulanger ('28), on the ground of lack of constancy for the Hasselbalch and the Raffin formulae, have objected to the whole idea of the exclusive restriction of ammonia for regulating urinary reaction and contend that its elimination is much more complex than would be the simple result of an acid-base regulating function of the kidney, being complicated, if in no other way, by the preformed ammonia of the blood, no matter how small.

In this connection it may be recalled that Bourquin ('24) found that when the output of urea is increased in caffeine diuresis there is an accompanying increase in the excretion of ammonia. Here the correlation could hardly be an artefact due to the simultaneous need of ammonia for acid neutralization, since caffeine produces a copious flow of gastric juice especially rich in HCl (Sollman, '18, p. 217) which, of itself, should lead to an alkaline urine. Also, it is well known that in liver disorders the output of ammonia is increased, since it "is not so completely transformed into urea before excretion" (Hawk and Bergeim, '26, p. 726). And, finally, although the demand for ammonia for neutrality regulation might run *pari passu* with protein catabolism and urea formation, it will be remembered that the correlation between ammonia and the other products of protein metabolism, amino acids, uric acid, creatinine, is negligible; suggesting a specific relation-

ship with urea. Also, it is worth noting that the correlation in the 24-hour urines (+0.49) is slightly more than twice as great as that in the short-period, basal ones (+0.23). This might suggest that during periods of exogenous increase of protein metabolism, when the concentration of protein split-products in the blood is high, ammonia fails to be quantitatively converted into urea and the two are excreted together; whereas under basal conditions there would be less opportunity for the escape of ammonia, except approximately that necessary for neutrality regulation, and so a lesser correlation with urea obtains.

To avoid misunderstanding it may be stated quite emphatically that no attempt is being made to disparage the importance of ammonia in neutrality regulation, but merely to argue, in so far as these data allow, for the possible appearance of ammonia (with urea) in the urine to some extent independently of this function. That a part of the ammonia (and, under basal conditions, perhaps most of it) is in the urine for this purpose may be inferred from the fact that aside from urea the only other significant correlation it has is with phosphates. This is in line with the parallel increase in phosphate and ammonia excretion that was observed by Campbell and Webster in night urines ('21) and urines of high acidity ('22).

Of the remaining correlations there is little to be said that would add to our understanding of metabolic origin or method of excretion. It may be seen that urea, creatinine and uric acid are closely correlated and that the latter, especially under the endogenous conditions represented by the basal urines, has its highest correlations with urea, creatinine and amino acids.

11. Correlations with total basal oxygen consumption

	+0.24.....	Oxygen, ammonia
Oxygen, urea	0.22.....	Oxygen, total nitrogen
	0.19.....	Oxygen, urea
	0.18.....	Oxygen, creatinine
Oxygen, total nitrogen	0.17.....	Oxygen, phosphorus
Oxygen, uric acid	0.14	
Oxygen, creatinine	0.11	

Oxygen, amino acid	0.08.....	Oxygen, amino acid
Oxygen, creatine	0.06	
Oxygen, ammonia	0.03.....	Oxygen, chloride
	0.01.....	Oxygen, uric acid
	-0.09.....	Oxygen, creatine

One of the chief interests in the work of which this is part was to define and, if possible, explain, day to day intra-individual variations of basal metabolic rate; and these urine analyses were primarily undertaken to discover what correlation might obtain between this and the prevailing level of protein metabolism as evidenced by urinary nitrogen. This has been referred to in the first report of the series (Griffith et al., '29, p. 616) where coefficients of correlation may be found for total and protein oxygen; the latter being derived from the total nitrogen figures of this paper. The accompanying tabulation (no. 11) gives also the correlation between total basal oxygen and the individual urinary constituents of which we have record.

While this work was in progress, Wishart ('28) brought out results dealing with the same subject, but more completely, in that the correlation was determined under conditions of wide variation of protein intake, as well as during the minor fluctuations of normal diet. The first gave large variations in both nitrogen excretion and basal metabolic rate in which the coefficients of correlation between the latter and total nitrogen, urea, ammonia, creatinine and uric acid ranged between $+0.71$ and $+0.76$; or, if a seeming lag of 3 days between nitrogen excretion and metabolic rate was allowed for, these coefficients rose to from $+0.76$ to $+0.85$. On normal diet, however, where errors of estimation are large in comparison with the actual changes in metabolism or excretion, the coefficients for metabolic rate and total nitrogen excretion were much reduced, being, for three subjects, between $+0.41$ and $+0.15$ and the latter scarcely greater than its probable error.

It is in this latter class that our results are comparable and confirmatory. Reference to our first report will show coefficients of correlation between basal oxygen and total nitrogen

of from $+0.01$ to $+0.37$ for the five subjects of our group, with a slightly higher average ($+0.22$) for short-period, basal than for 24-hour excretion ($+0.17$; see, also, tabulation no. 11, above).

Although therefore, the correlation between basal oxygen consumption and total nitrogen excretion is small when calculated from the day-to-day variations on normal diet, there is good reason to believe the soundness of Wishart's opinion that, under these conditions, the fundamental correlation is merely obscured by errors of estimation which then become large in comparison with the variations of the functions being determined. For it will be apparent in later sections when we come to deal with averages, as, for example, during menstrual or seasonal cycles, there is a close parallelism between the curves for nitrogen excretion and metabolic rate; i.e., the plus and minus errors of analysis cancel out and the correlated variations become more evident.

On account of its apparently close relationship to active protoplasmic mass, creatinine is the only nitrogenous excretory product, which, prior to the work of Wishart and of ourselves, has received attention as a possible index of the rate of cellular metabolism. Wishart's data and our own show that, as far as intraindividual variations are concerned, this substance has nothing to recommend it above urea or total nitrogen; and to this extent confirm the earlier negative findings of Palmer, Means and Gamble ('14) and Eichelberger ('25).

In conclusion, it is interesting to compare the correlation of oxygen consumption with excretion computed from analysis of 24-hour and short-period, basal urines, respectively. Thus, urea from either type of urine shows practically the same degree of correlation with the oxygen consumption; and the same, or rather similar lack of correlation, is evidenced by the remaining components of both urines, with the exception of total nitrogen and ammonia. The total nitrogen of the basal urines is correlated in higher degree with oxygen consumption than is that of the 24-hour samples; and this dif-

ference, though perhaps too small here to be of much significance, deserves attention as indicative of the much sharper contrast of the same type that will be apparent when we come to compare the seasonal variations of both functions. The most striking reversal, however, is shown by ammonia. There is no correlation between it and oxygen consumption and it stands the very lowest of the series when computed from analysis of 24-hour urines. On the other hand, its excretion in the short-period, basal urines shows higher correlation with oxygen utilization than any other component. Until this can be tested by further work, it is futile to speculate as to its meaning. If it is taken to indicate that variations in metabolic rate are related to fluctuations in acid-base equilibrium rather than specifically to variations in protein metabolism, then the lack of correlation with 24-hour ammonia excretion might be explained according to the argument of a preceding page which tended to show that the relation of ammonia to neutrality regulation is obscured in 24-hour samples by the operation of other factors. But its chief interest at the moment is as a challenge to further investigation.

II. THE EFFECT OF MENSTRUATION

The data from urines obtained during menstruation and during the first, second, third and fourth weeks of the intermenstrual period have been grouped and their averages are given in table 2. The grand averages for the three subjects are shown in figure 1.

Excepting creatine and uric acid (not included in the graph), all of the excretion products for which we have analyses appear to vary during the menstrual cycle according to one of the following two plans: 1) excretion lowest at about the time of menstruation and maximum during the intermenstrual period. Most of the substances belong to this group which includes: urea, creatinine, amino acids, total and undetermined nitrogen, sugar, water and, very probably, chlorides. The occurrence of maximum excretion during the intermenstrual period is well established by all of the indi-

TABLE 2

The effect of menstruation on urinary excretion. Averages of the determinations made during the menstrual periods and during the first, second, third and fourth weeks of the inter-menstrual periods. The data for E.F. and G.H. are derived from 24-hour, and those for K.L., from short-period, basal, morning urines. For purposes of comparison all are expressed as 24-hour values. The numbers in parentheses are the number of observations on which each average is based

FUNCTION	SUBJECT	MENSTRUAL PERIOD	WEEKS FOLLOWING MENSTRUATION			
			First	Second	Third	Fourth
Volume, cubic centimeter per 24 hours	E.F.	836 (7)	1026 (12)	907 (9)	1063 (10)	863 (10)
	G.H.	591 (7)	665 (11)	663 (9)	683 (10)	625 (9)
	K.L.	1921 (7)	2631 (20)	2424 (18)	2679 (18)	1752 (18)
	Average	1116 (21)	1680 (43)	2219 (36)	1728 (38)	1237 (37)
Specific gravity	E.F.	1.0236 (7)	1.0200 (12)	1.0202 (9)	1.0226 (10)	1.0205 (10)
	G.H.	.0260 (6)	.0256 (11)	.0282 (9)	.0243 (10)	.0259 (9)
	K.L.	.0131 (7)	.0106 (19)	.0141 (17)	.0114 (17)	.0188 (18)
	Average	1.0206 (20)	1.0172 (42)	1.0193 (35)	1.0179 (37)	1.0210 (37)
Urea-N, grams per 24 hours	E.F.	4.626 (7)	5.546 (12)	5.249 (9)	5.900 (10)	5.609 (10)
	G.H.	4.894 (7)	5.515 (11)	5.799 (9)	5.275 (10)	5.672 (9)
	K.L.	6.422 (7)	6.343 (19)	7.281 (18)	8.219 (18)	6.633 (18)
	Average	5.314 (21)	5.899 (42)	6.402 (36)	6.834 (38)	6.122 (37)
NH ₃ -N, grams per 24 hours	E.F.	0.509 (7)	0.477 (12)	0.444 (9)	0.505 (10)	0.495 (10)
	G.H.	.251 (7)	.321 (10)	.242 (9)	.288 (9)	.287 (9)
	K.L.	.197 (7)	.196 (19)	.198 (18)	.215 (18)	.261 (18)
	Average	0.319 (21)	0.309 (41)	0.269 (36)	0.311 (37)	0.330 (37)
Creatinine-N, grams per 24 hours	E.F.	0.370 (7)	0.391 (12)	0.380 (9)	0.404 (10)	0.365 (10)
	G.H.	.293 (7)	.334 (11)	.331 (9)	.325 (10)	.325 (9)
	K.L.	.357 (6)	.344 (18)	.354 (18)	.373 (17)	.372 (18)
	Average	0.339 (20)	0.355 (41)	0.355 (36)	0.368 (37)	0.358 (37)
Creatine-N, grams per 24 hours	E.F.	0.046 (6)	0.040 (12)	0.043 (9)	0.051 (10)	0.052 (10)
	G.H.	.046 (7)	.044 (11)	.053 (9)	.035 (10)	.051 (9)
	K.L.	.015 (5)	.027 (19)	.027 (16)	.021 (17)	.015 (18)
	Average	0.037 (18)	0.035 (42)	0.038 (34)	0.033 (37)	0.034 (37)
Uric acid-N, grams per 24 hours	E.F.	0.179 (7)	0.153 (12)	0.145 (9)	0.167 (10)	0.150 (10)
	G.H.	.109 (7)	.137 (11)	.121 (9)	.120 (10)	.136 (9)
	K.L.	.148 (6)	.156 (19)	.152 (18)	.161 (17)	.141 (18)
	Average	0.145 (20)	0.150 (42)	0.143 (36)	0.151 (37)	0.142 (37)
Amino acid-N, grams per 24 hours	E.F.	0.100 (7)	0.111 (12)	0.135 (9)	0.113 (10)	0.106 (10)
	G.H.	.083 (7)	.099 (11)	.094 (9)	.093 (10)	.078 (9)
	K.L.	.088 (6)	.094 (19)	.094 (18)	.109 (16)	.089 (16)
	Average	0.091 (20)	0.100 (42)	0.104 (36)	0.105 (36)	0.091 (35)
Total N, grams per 24 hours	E.F.	6.210 (7)	7.094 (12)	7.063 (9)	7.583 (10)	7.140 (10)
	G.H.	6.147 (7)	6.902 (11)	7.163 (9)	6.801 (10)	7.005 (9)
	K.L.	7.613 (7)	7.859 (20)	8.369 (18)	9.559 (18)	7.953 (18)
	Average	6.657 (21)	7.400 (43)	7.741 (36)	8.313 (38)	7.503 (37)
Rest N, grams per 24 hours	E.F.	0.354 (7)	0.416 (12)	0.721 (9)	0.448 (10)	0.406 (10)
	G.H.	.470 (7)	.396 (10)	.486 (9)	.499 (10)	.468 (9)
	K.L.	.356 (6)	.351 (18)	.396 (16)	.431 (16)	.407 (14)
	Average	0.396 (20)	0.382 (40)	0.506 (34)	0.454 (36)	0.423 (33)
Sugar, grams per 24 hours	E.F.	0.428 (7)	0.484 (12)	0.516 (9)	0.494 (9)	0.465 (10)
	G.H.	.468 (7)	.458 (11)	.690 (9)	.534 (10)	.512 (9)
	K.L.	.274 (6)	.344 (19)	.366 (18)	.366 (17)	.346 (18)
	Average	0.396 (20)	0.414 (42)	0.484 (36)	0.445 (36)	0.419 (37)
Organic acids, cubic centimeter, N/10	E.F.	581 (6)	553 (11)	581 (8)	573 (10)	558 (10)
	G.H.	442 (7)	373 (11)	457 (10)	418 (9)	375 (7)
	K.L.	489 (4)	481 (17)	432 (15)	542 (15)	464 (11)
	Average	502 (17)	472 (39)	498 (33)	518 (34)	475 (28)
Total acid, cubic centimeter, N/10	K.L.	138 (6)	104 (17)	119 (16)	172 (17)	209 (17)
Phosphorus, grams per 24 hours	K.L.	0.581 (6)	0.508 (17)	0.567 (18)	0.667 (17)	0.645 (18)
Chlorides, grams per 24 hours	K.L.	8.640 (6)	11.300 (18)	9.852 (18)	9.847 (16)	8.532 (18)

vidual curves, and, with infrequent exception, it occurs at the middle or during the latter half, so that the average curves of figure 1 are well authenticated in this respect. The apparent exception of chloride is probably not of serious import, since this is but the individual curve for subject K.L., for whom, alone, this was determined, and the established correlation between chloride and water would no doubt be evidenced in an average variation of the former, were more

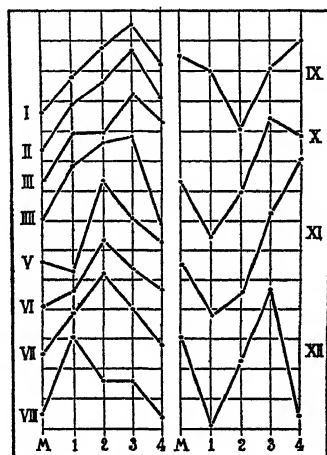


Fig. 1 The effect of menstruation on urinary excretion. The curves are derived from the grand averages of table 2. M, 1, 2, 3, and 4 refer to the menstrual period and the first, second, etc., weeks of the intermenstrual period, respectively. I, urea; II, total N; III, creatinine; IV, amino acid; V, undetermined N; VI, sugar; VII, volume; VIII, chloride; IX, ammonia; X, phosphate; XI, total acid; XII, organic acid.

data available, similar to the curve as we have it for the latter. The exact incidence of the lowest point is similarly uncertain. In most cases it occurs during the menstrual period, but there are enough instances of it coming just before or just after to make it impossible to say more than that it occurs at about the time of menstruation.

2) The second pattern of variation is represented by ammonia, phosphate, and total and organic acid. In these the minimal excretion occurs during the first half or middle of

the intermenstrual period and rises to a maximum toward its close. Again, individual differences prevent placing the maxima and minima with greater precision and it must be remembered that phosphate and total acid were determined on only one subject, K.L., so that the curves for these in figure 1 are individual and not averages. But, taken together, they show sufficient similarity to satisfy the expected physiological correlation and so strengthen and support one another.

Discussion. On the whole these results are in agreement with such previous work as we have been able to find. Like us, Rose ('17) and Stearns and Lewis ('21) were unable to find any connection between menstruation and the output of creatine. A retention of nitrogen during menstruation with a compensatory increase in output later was first reported by von Schrader (1894) and has since been confirmed by Murlin ('10, '11), Sherman, Gillett and Pope ('18) and Gillett, Wheeler and Yates ('19). Our results are not in agreement, however, with the report of Krause ('11) that after menstruation the distribution of nitrogen in the urine is changed so as to increase ammonia and undetermined nitrogen, while urea decreases. As shown above, we find this true only for the rest nitrogen, while urea increases and ammonia, though less certainly, decreases. And, although our data for ammonia are not too conclusive, the variation we have observed is similar to that previously reported by Bond ('22).

As regards water and chloride excretion, Heilig has reported ('24) that when water and chloride are ingested the amount excreted is only half as great during menstruation as during the intermenstrual period—a finding quite in accord with our result.

The failure of Sherman, Gillett and Pope ('18) and of Gillett, Wheeler and Yates ('19) to find a menstrual variation in the output of phosphates, deprives us of confirmation for the definite variation shown by our data. However, it must be remembered that these were obtained on only one subject and from basal, short-period, rather than 24-hour urines, which makes the result none too certain or too rigorously comparable to the previous findings.

In conclusion, it may not be inappropriate to call attention to concurrent variation of other functions which appear to fit consistently with the excretion described here and which by their interrelationship strengthen and support each other.

Thus, the basal metabolic rate, oral temperature (Griffith et al., '29 a), and pulse rate (ibid., '29 b) are, like the total nitrogen and most of its partition products, lowest during or just after the period and rise during the intermenstruum to their highest values just preceding the onset of menstruation.

At the time of our previous publication there was evidence for such a variation of the metabolic rate and it has since been confirmed by Matters ('29), Wible ('31), McClendon, Myrick, Conklin and Wilson ('31) and, although they refuse to so interpret their data, Sandiford, Wheeler and Boothby ('31). We were unaware at the time that our data merely confirmed previous findings of Cullis, Oppenheimer and Ross ('22) for temperature; and of these last and, also, Moore and Cooper ('23) and Truesdell and Croxford ('26) for pulse rate; and are happy to correct these unintentional omissions at this time.

Boothby and his co-workers have just been referred to as refusing to interpret the variations of metabolic rate which they have obtained as intrinsic menstrual effects. The ground of their contention is that the variations are small and no greater than might occur fortuitously. This is undoubtedly true and it is as true of any of the other variables, taken separately, as it is of the metabolic rate itself. It is even possible that the metabolic rate, pulse rate and temperature might vary concurrently but fortuitously, not as an intrinsic menstrual effect, but merely the reflection at the moment of the degree of relaxation, repose or apprehension prevailing at the time of the determination. It is difficult, or so it appears to us, to fit the data regarding nitrogen excretion into such a scheme of transient instability. Even the nitrogen of the short-period urines excreted during the time of the metabolism determination would hardly be affected by such conditions prevailing at the time; but the result was the

same with the 24-hour samples collected during the day preceding the metabolism determination. In both cases the nitrogen excretion is lowest during menstruation and highest toward the end of the intermenstrual period. And in the light of the correlation which Wishart, particularly, has established between nitrogen excretion and metabolic rate, the variation which now appears to be established for the latter would seem to be precisely as demanded.

To complete the picture, we may refer to the variation we have observed in alveolar carbon dioxid percentage and pressure, the blood carbon dioxid capacity and pulmonary ventilation (Griffith et al., '29 c and '29 d). These have been referred to in our preceding report (*ibid.*, '29 d) and need not be discussed in detail here. Suffice it to repeat that the variation of alveolar carbon dioxid was the most clear-cut and indubitable of any menstrual effect we have observed; and in the preceding report it was shown that the expected physiological relationship obtained between it and the variations of alkali reserve and pulmonary ventilation. Finally, it may be added that ammonia, phosphate and total acid of the urine appear to be related logically with the alkali reserve, being excreted at a minimum (during the early part of the intermenstrual period) when this is highest. We have, then, in the alveolar carbon dioxid pressure, alkali reserve, pulmonary ventilation and acid excretion four independent measurements of the same fundamental physiological state, and their concurrent variation would seem to establish beyond all doubt the occurrence of deep-seated metabolic changes during the menstrual cycle. It is not unreasonable to hope that further work may establish a relationship between the metabolic group (oxygen consumption, nitrogen excretion, pulse rate and temperature), on the one hand, and these measures of the acid-base balance, on the other.

III. SEASONAL VARIATION

The conclusions of this section are based on the grand averages of table 3, which are shown graphically in figure 2. It may be recalled that four subjects (A.B., C.D., E.F., and G.H.) each furnished a 24-hour sample approximately once a week during 1925; so, for this year the grand averages for each month, on which the curves of figure 2 are based, are derived from approximately sixteen determinations apiece. During 1926, three subjects (A.B., C.D., and K.L.) furnished short-period, basal, morning urines usually twice a week, so the grand averages for this year are based on approximately twenty-four analyses per month.

In the discussion of these results, it seems necessary to distinguish between the 24-hour and the short-period, basal urines. For, with the exception of organic acid and possibly ammonia, which appear to vary somewhat in the same degree in both types, there is too little similarity between them to permit direct comparison. Thus, confining ourselves principally to total nitrogen, though what is said of it applies in varying degree to its partition products, there is no evidence that it undergoes any definite seasonal variation in the 24-hour urines. On the other hand, its variation in the basal urines is remarkably clear-cut and uniform, being maximum in the winter and (if we neglect the one irregularly low point in November) minimum in the early fall. And, in so far as they seem to follow any definite pattern at all, the same seems to be true of urea, creatinine, creatine, uric acid, amino acids, undetermined nitrogen, volume and chlorides. Phosphates are too irregular to permit any definition. And total acid (omitting the irregularly low point for October), organic acid and ammonia appear to vary according to a pattern that will be discussed in more detail in the following report and in which the lowest values occur in the spring and the highest in the late summer or autumn.

TABLE 3

Monthly averages. The data for 1925 are derived from 24-hour urines and are so given; those for 1926 were obtained from short-period, basal, morning urines and are all computed to values per 120 minutes which was approximately the average time of collection (see last entry of table 1). During 1925, each subject furnished one 24-hour sample approximately once a week; so the individual monthly averages are based on approximately four, and the grand averages on approximately sixteen determinations each. During 1926, each subject furnished, on the average, two short-period, basal, morning samples per week; so for this year the individual averages are based on approximately eight, and the grand averages on approximately twenty-four determinations each.

	VOLUME	SPECIFIC GRAVITY	URIC ACID M	MG. M	CREATININE M	CREATININE M	URIC ACID M	AMINO ACID M	TOTAL N	REST N	SUGAR	ORGANIC ACID (0.1 N)	CORRECTED ORGANIC ACIDS (0.1 N)	TOTAL ACID (0.1 N)	PHOSPHATE	CHLORIDE
Cubic centimeters and grams per 24 hours																
Total average	864	1.023	5.99	.392	.438	.047	1.62	.091	7.76	.68	.596	540.2	348			
(A.B.,	853	24	6.95	.488	.456	.043	.149	.095	8.59	.50	.573	486.0	287			
C.D.,	976	24	7.22	.399	.439	.062	.163	.110	8.95	.58	.545	498.4	297			
E.F.,	986	22	6.32	.410	.423	.052	.131	.086	7.83	.43	.541	425.5	319			
G.H.)	993	22	6.12	.411	.388	.051	.123	.108	7.62	.49	.498	505.3	329			
July	868	23	6.59	.390	.424	.042	.140	.118	8.45	.62	.470	581.5	404			
Aug.	916	23	7.12	.435	.464	.051	.144	.135	8.87	.52	.537	592.4	386			
Sept.	985	22	6.68	.450	.401	.058	.152	.096	8.23	.40	.510	521.1	339			
Oct.	941	19	6.75	.427	.401	.028	.154	.082	8.29	.37	.610	538.8	362			
Nov.	1058	22	7.04	.438	.472	.033	.180	.105	8.67	.41	.588	544.4	336			
Dec.	945	26	7.31	.462	.437	.042	.182	.105	8.96	.44	.587	489.2	282			
1926 Jan.	930	28	7.19	.473	.460	.035	.198	.110	9.14	.51	.587	508.4	292			
Cubic centimeters and milligrams per 120 minutes																
(A.B.,	157	1.016	668	33.4	40.7	2.2	18.2	9.7	810	44	83.3	44.3	29.3	9.91	46.4	1074.6
C.D.,	211	13	700	25.1	39.2	2.9	15.0	8.4	804	49	44.7	39.6	22.0	7.77	42.1	1058.7
E.F.,	169	15	677	26.8	37.1	2.8	15.4	7.8	774	46	32.7	39.3	22.3	10.65	46.6	1020.2
G.H.)	191	13	670	26.5	37.9	2.1	16.2	9.2	784	45	33.3	42.7	21.4	9.76	46.0	1014.4
July	207	13	638	29.0	37.8	1.9	15.1	8.1	772	49	30.2	37.7	20.8	11.83	46.0	980.5
Aug.	189	16	639	26.2	37.1	1.7	15.5	8.7	751	29	30.0	44.5	27.4	12.63	40.1	913.4
Sept.	107	18	619	33.0	37.5	0.9	15.4	7.5	747	29	30.0	42.5	26.2	13.20	40.0	782.5
Oct.	164	15	631	24.2	38.4	1.4	14.8	9.7	731	26	29.5	43.9	26.3	12.21	40.8	1012.5
Nov.	186	15	625	27.4	38.9	1.0	15.5	9.0	735	31	30.0	45.7	28.0	4.84	42.8	1020.3
Dec.	151	17	622	26.3	38.9	1.1	15.4	8.0	729	34	29.9	43.7	26.6	10.84	45.7	834.1
1927 Jan.	140	18	611	26.6	38.6	0.7	15.3	8.7	747	48	28.6	49.2	31.4	10.84	32.2	821.5
	139	20	650	31.3	38.7	1.6	16.4	9.0	788	41	28.6	50.3	32.6	13.88	30.3	844.8

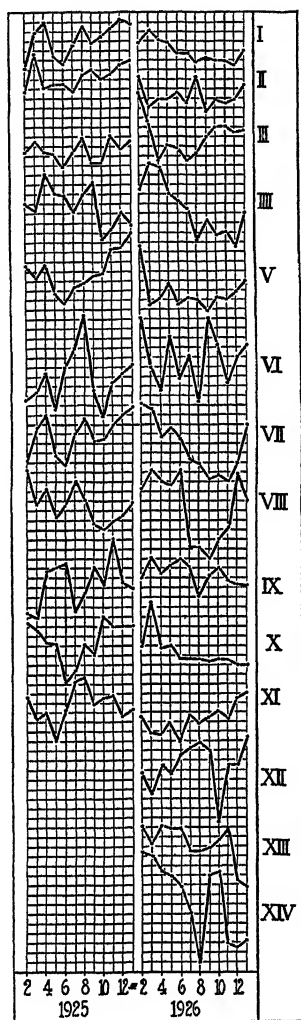


Fig. 2 Seasonal variation in urinary excretion. The curves are derived from the grand averages of table 3. Those for 1925 represent 24-hour and those for 1926, short-period, basal, morning excretion. I, urea; II, ammonia; III, creatinine; IV, creatine; V, uric acid; VI, amino acid; VII, total N; VIII, undetermined N; IX, volume; X, sugar; XI, organic acid; XII, total acid; XIII, phosphate; XIV, chloride.

Discussion. Denis and Borgstrom have reported ('24) that nitrogen excretion and therefore ingestion, of medical students in New Orleans, is lower than the average for colder regions; and, further that it is diminished in the warm as compared with the colder months. The first, which they regarded as giving probability to the second, has since been refuted by Youngburg and Finch ('26), Beard ('27) and Brooks ('29), who have shown that the average excretion of medical students is very similar in western New York, northern Ohio and North Carolina to that reported by Denis and Borgstrom for semi-tropical New Orleans; and, in addition, they have presented evidence seriously discrediting seasonal variation. Such, likewise, is the testimony of our own data for daily excretion, to which the above applies.

On the other hand, we know of no previous data comparable to that which we present here for short-period, basal urines and which we interpret as showing a very definite seasonal variation. These must represent very closely the endogenous metabolism; and it is a matter of very great interest that the type of variation which this shows (minimum in late summer or early fall) is identical with that which we have previously described for body weight, basal oxygen consumption (Griffith et al., '29 a) and basal pulse rate (ibid., '29 b). This must have the very considerable significance of indicating that the variation of metabolic rate which was characteristic of this group was not due to parallel variation in protein ingestion which would be chiefly responsible for the form of the curves for 24-hour excretion. Which is cause and which effect cannot at present be determined. All that can be said is that our data establish a closely correlated seasonal variation for basal nitrogen excretion and oxygen utilization.

An exception to the above is ammonia. As was mentioned previously, this, together with total and organic acid, seems to vary according to another plan which is widely represented among the physiological functions we have studied and which will be discussed in the following paper of this series.

SUMMARY

This is a continuation of reports already made (Griffith et al., '29) for basal metabolic rate and respiratory and cardiovascular functions in an attempt to define the variability and correlation of intra-individual variations over a course of time.

This report deals with the excretion of the principal nitrogen components of the urine, together with sugar, organic and total acid, phosphate and chloride.

Comparison is provided of the rate of excretion in 24-hour urines with that of short-period, morning urines under basal conditions.

By their coefficients of variation the relative constancy of excretion of these urinary components is established, 1) with respect to each other; and, 2) with respect to the other functions previously reported upon. As usual, the women of the group prove to be more variable than the men.

Coefficients of correlation are given which provide a measure of the interrelationship between, 1) the various urinary constituents themselves and, 2) between them and the simultaneous variations of basal metabolic rate.

Menstruation clearly affects the excretion of all these substances, with the exception, perhaps, of creatine and uric acid. Urea, creatinine, amino acid, total and undetermined nitrogen, volume, sugar and chloride are excreted in minimal amounts at about the time of menstruation and rise to a maximum in the latter half of the intermenstrual period. This is the type of variation previously observed in the women of this group for the oral temperature, pulse and basal metabolic rate. Ammonia and possibly organic and total acid and phosphate are roughly the reciprocal of this and show minimal excretion toward the middle of the intermenstrual period. This is shown to correlate with the previous findings in respect to menstrual variations in alveolar carbon dioxide pressure, blood alkali reserve and pulmonary ventilation. These correlations provide an integrated and more complete picture of physiological variation during menstruation than has been available hitherto.

There was apparently no seasonal variation for most of the components of the 24-hour urines. On the other hand, the total nitrogen and most of its partition products and volume and chloride of the short-period, basal urines are definitely excreted at a minimum during the late summer or early fall, with maxima in the winter. This is precisely the type of seasonal variation shown by the averages for this same group for body weight and basal pulse and metabolic rate. Exceptions to this type of variation are total and organic acids and, possibly, ammonia, which seem to be excreted at a minimum in the spring and maximum in the late summer or early fall, a pattern observed by many of the functions we have studied and which will be discussed in greater detail in the following report of this series.

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STUDIES IN HUMAN PHYSIOLOGY

VI. VARIATIONS IN BLOOD CHEMISTRY OVER LONG PERIODS OF TIME, INCLUDING THOSE CHARACTERISTIC OF MENSTRUATION

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TWO FIGURES

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Preceding reports of this series have described the intra-individual variation of basal metabolic rate, pulse rate and blood pressure, alveolar air and blood gas capacity, pulmonary ventilation (Griffith et al., '29) and urinary excretion (Pucher et al., '34) of five normal, adult subjects who were under observation during the 2-year period from February, 1925, to February, 1927. It is the purpose of this report to record observations on the composition of the blood of four of these subjects.

Two of the subjects, A.B. and C.D., were men and served continuously throughout the 2-year period; the other two, E.F. and G.H., were young women who were under observation during the first year (February, 1925, to February, 1926) only. For further details the first report of the series should be consulted.

From each subject, during the period of his or her service, 15 to 20 cc. of blood were taken from an arm vein approximately once a week. This blood was drawn, without disturbing the subject, and while in the basal state, either just preceding or just following the collection of the expired air for determination of the basal metabolic rate. For this pur-

pose the subject reported to the laboratory, without breakfast, between 8 and 9 A.M., and lay down. After a preliminary rest period of from 30 to 45 minutes the blood sample was taken, or the metabolism period run and the sample then obtained. In either event the blood composition may be taken to represent the basal condition.

As explained in the previous reports, during the day preceding the metabolism determination and, therefore, the taking of the blood sample, no meat was eaten. This single departure from otherwise unrestricted, normal conditions was made primarily to avoid any excessive stimulation of the metabolic rate by an occasional plethora of protein metabolites. Since, however, the subjects ate otherwise according to desire of milk, eggs and other meat substitutes it is hardly to be supposed that the nitrogen components of the blood would be abnormally affected.

The methods of analysis were standard and may be found most conveniently in the following citations from Hawk and Bergeim, *Practical Physiological Chemistry*, 9th edition:

- Non-protein nitrogen; micro-Kjeldahl, page 368.
- Urea nitrogen; aeration process, page 371.
- Amino acid nitrogen; Folin process, page 380.
- Uric acid nitrogen; Benedict method, page 377.
- Total creatinine; page 376.
- Sugar; Folin and Wu, page 381.
- Cholesterol; Myers and Wardell modification, page 391.
- Phosphate, inorganic; Fiske and Subbarow, page 403.
- Calcium; Clark-Collip, page 408.
- Chloride (Na); Whitehorn, page 401.
- Corpuscles; Hematocrit.

I. STATISTICS

1. *Averages.* Table 1 gives the maximum and minimum, mode, arithmetical mean, standard deviation, coefficient of variation and number of observations for each blood constituent for each individual. Of these values, those for non-protein and urea nitrogen, sugar, calcium and chloride are within the usually accepted normal limits (Hawk and Bergeim, '26, loc. cit., p. 357); uric acid, total creatinine and

TABLE 1

Statistics of intra-individual variation of blood composition

FUNCTION	SUBJECT	MINIMUM AND MAXIMUM	MODE	ARITHMETICAL MEAN	STANDARD DEVIATION	COEFFI- CIENT OF VARIATION	NUMBER OF OBSERVA- TIONS
Non-protein nitrogen. Milligrams, per cent	A.B.	21-38	28	30.0±0.3	3.72	12.4	91
	C.D.	20-37	27; 31	29.4±0.3	4.04	13.8	91
	E.F.	19-31	23; 24	23.9±0.3	2.73	11.4	46
	G.H.	20-40	29	28.6±0.4	4.29	15.0	43
	Average			28.0	3.69	13.2	271
Urea nitrogen. Milligrams, per cent	A.B.	7-18	13	12.5±0.1	1.97	15.8	91
	C.D.	6-17	11	11.2±0.2	2.28	20.3	95
	E.F.	5-12	7	8.2±0.2	1.98	24.2	46
	G.H.	8-17	9; 11	12.0±0.3	2.53	21.1	42
	Average			11.0	2.19	20.4	274
Amino acid nitrogen. Milligrams, per cent	A.B.	6.5-12.5	8.5	8.8±0.1	1.04	11.8	93
	C.D.	6.0-12.5	9.5	9.0±0.1	1.12	12.5	92
	E.F.	6.0-11.5	8.5	8.4±0.1	1.33	15.8	46
	G.H.	6.0-12.5	8.5; 9.5	9.0±0.2	1.58	17.6	43
	Average			8.8	1.27	14.5	274
Uric acid nitrogen. Milligrams, per cent	A.B.	0.6-1.4	0.9	0.96±0.01	0.17	17.5	90
	C.D.	0.8-2.3	1.3	1.32±0.02	0.22	16.6	93
	E.F.	0.7-1.6	1.0	1.13±0.02	0.19	16.4	46
	G.H.	0.6-1.7	0.9	1.05±0.02	0.23	22.3	42
	Average			1.12	0.21	18.3	271
Total creatinine nitrogen. Milligrams, per cent	A.B.	1.4-2.2	1.90	1.84±0.02	0.17	9.4	48
	C.D.	1.4-2.2	1.75; 1.80	1.77±0.02	0.19	10.5	50
	E.F.	0.9-2.3	1.80	1.78±0.02	0.23	12.9	47
	G.H.	1.4-2.4	1.80	1.88±0.02	0.17	9.1	45
	Average			1.82	0.19	10.5	190
Rest nitrogen. Milligrams, per cent	A.B.	1-14	6	6.60±0.2	2.52	38.2	87
	C.D.	3-16	7	7.20±0.2	2.67	37.0	88
	E.F.	1-15	5	4.60±0.3	2.99	64.9	46
	G.H.	1-10	3; 4	4.90±0.3	2.50	50.9	42
	Average			5.9	2.65	47.8	263
Sugar. Milligrams, per cent	A.B.	72-117	91; 92	89.4±0.5	7.37	8.3	92
	C.D.	70-117	103	99.5±0.8	11.90	11.9	93
	E.F.	73-116	93; 94; 95	98.3±0.8	7.62	8.2	46
	G.H.	79-127	97	100.8±1.0	9.47	9.4	41
	Average			95.8	9.09	9.5	272
Cholesterol. Milligrams, per cent	A.B.	70-130	110	106.9±1.0	12.20	11.4	90
	C.D.	70-155	115	112.7±1.1	13.40	11.9	90
	E.F.	85-135	110	109.1±1.1	10.40	9.5	44
	G.H.	95-150	110-120	116.2±1.1	10.50	9.1	42
	Average			111.3	11.62	10.5	266
Inorganic phosphate. Milligrams, per cent	A.B.	1.6-3.8	2.6	2.78±0.03	0.39	13.9	82
	C.D.	1.6-3.6	2.8	2.72±0.03	0.37	13.7	87
	E.F.	2.5-4.1	3.1	2.83±0.04	0.39	11.1	45
	G.H.	2.2-3.7	3.0	3.33±0.03	0.30	9.6	41
	Average			2.92	0.37	12.2	255
Calcium. Milligrams, per cent	A.B.	8.6-11.6	10.2; 10.4	10.19±0.04	0.59	5.7	81
	C.D.	9.1-12.3	10.3	10.32±0.04	0.57	5.5	87
	E.F.	9.0-11.9	9.9	10.10±0.06	0.56	5.6	44
	G.H.	8.9-11.2	10.2	10.23±0.06	0.53	5.2	39
	Average			10.22	0.57	5.5	251
Sodium chloride Milligrams, per cent	A.B.	455-545	475	489.2±1.7	17.45	3.6	81
	C.D.	460-610	475	496.6±1.8	24.75	5.0	84
	E.F.	455-550	475	499.1±2.7	25.25	5.2	40
	G.H.	475-565	495; 530	521.4±2.6	24.15	4.6	40
	Average			501.6	22.90	4.6	245
Per cent corpuscles	A.B.	37-54	45	45.7±0.2	3.12	6.8	92
	C.D.	42-55	46	47.3±0.2	3.03	6.4	90
	E.F.	37-48	42; 43	42.8±0.2	2.22	5.2	45
	G.H.	33-43	40	38.9±0.3	2.56	6.6	43
	Average			43.6	2.74	6.3	270

undetermined nitrogen, cholesterol and inorganic phosphate are slightly lower, whereas amino acid nitrogen is higher than the figures commonly given. Such differences as exist are, however, not large and (except the higher values for amino acids) may be attributable to the basal state, for which they are the most accurate definition at present available and as such perhaps hold whatever interest may attach to them.

2. *Variability.* One of the primary objectives in the study of which this report is the last, has been to ascertain the constancy with which the functions that have been investigated are regulated over a period of time. For this purpose we have employed the coefficient of variation and a summary of this datum for the blood constituents (taken from table 1) follows:

<i>Coefficients of variation</i>			
<i>Blood constituent</i>	<i>Men</i>	<i>Women</i>	<i>Average</i>
Chlorides	4.3	4.9	4.6
Calcium	5.6	5.4	5.5
Per cent corpuscles	6.6	5.9	6.3
Sugar	10.1	8.8	9.5
Cholesterol	11.7	9.3	10.5
Total creatinine nitrogen ...	10.0	11.0	10.5
Inorganic phosphate	13.8	10.6	12.2
Non-protein nitrogen	13.1	13.2	13.2
Amino acid nitrogen	12.2	16.7	14.5
Uric acid nitrogen	17.1	19.4	18.3
Urea nitrogen	18.1	22.7	20.4
Undetermined nitrogen	37.6	57.9	47.8

A summary of the coefficients of variation for the functions previously reported upon, exclusive of those for urinary excretion, can be found in the fourth report of this series (Griffith et al., '29 d, p. 569) and for the excretion of the commoner urinary constituents in the preceding one (Pucher et al., '34). Reference to these will show that of twenty-seven measurements, including metabolic, cardio-vascular and respiratory functions, the coefficients of variation ranged from 0.5 to 12.9. Urinary excretion was uniformly less constant, varying, except for specific gravity (0.6) and creatinine under basal conditions (9.6), from 15.4 to 92.3. Objection could have been made that the higher variability of the uri-

nary constituents might have been due, at least in part, to greater error inherent in the methods of analysis involved in their determination. This suspicion is considerably allayed by the data given here for the blood constituents. The determination of these involved errors similar to those of urine analysis, yet, with the exception of urea, the greater constancy of the blood composition is apparent from the following summary:

Coefficients of variation

	<i>Blood</i>	<i>Urine</i>
Chloride	4.6	33.6
Sugar	9.5	22.9
Inorganic phosphate	12.2	29.4
Total nitrogen	13.2	16.6
Amino acids	14.5	28.0
Uric acid	18.3	25.5
Urea	20.4	18.8
Undetermined nitrogen	47.8	52.6

This is simply a statistical statement of a fact known long since that one of the important mechanisms for maintaining the composition of the blood within definite limits is variable excretion in the urine.

We know of only one previous attempt to define the intra-individual variability of the blood constituents; this is by Hammett ('20) who used the average deviation from the mean as the measure of variability. Our evidence is an almost exact confirmation of his, the only exception being that he found sugar more variable than creatinine or total non-protein nitrogen, whereas with us it is less variable than any of the nitrogenous components.

Another matter that has consistently forced itself upon the attention in connection with the functions previously reported upon has been the prevailing greater variability shown by the women subjects of the group. Thus of the total of forty-two variables for which we have the data, the coefficients of variation were greater for the women than the men in thirty. It may be seen from the summary preceding the one given just above that this ratio does not obtain among the constituents of the blood reported on here. Of these only

amino acid, uric acid, total creatinine and urea are slightly, and undetermined nitrogen significantly more variable in the women than in the men.

3. *Correlations.* In conclusion, one of the objectives originally in mind when this study was undertaken was to determine the extent of day-to-day intra-individual variations of the basal metabolic rate and in how far these might be caused by, or at least correlated with simultaneous variation of other functions. In so far as the blood constituents reported on here are concerned there are no significant interrelationships between their variation and that of the simultaneously determined basal metabolic rate. Inspection of the correlation tables indicated in each case such complete lack of relationship that computation of the coefficients seemed an unnecessary expenditure of effort.

II. MENSTRUAL VARIATION

Blood samples were obtained from the two women subjects, E.F. and G.H., as a rule once a week during the year 1925. Data are available, therefore, for approximately twenty-four menstrual cycles. Those determinations made during the menstrual periods and each of the four weeks following have been grouped and the averages are given in table 2 and shown graphically in figure 1.

The only unequivocal variation shown by these data is by uric acid and chloride. Both are exactly alike with highest values during menstruation and a continuous fall during the intermenstrual period.

Except for the high value shown by E.F., in the fourth week, total creatinine would just as definitely follow the above pattern.

Also, except for the low value shown by E.F., during menstruation, cholester \acute{o} l varies similarly in both subjects. The highest value is reached the week after menstruation and, granting the exception, the lowest 2 weeks later. Sugar, though more doubtful, is alike for both subjects, similarly lowest during the third intermenstrual week and probably highest at about the time of menstruation.

TABLE 2

Variation of blood composition during the menstrual cycle; averages of the observations made during menstruation and during each week following; the numbers in parentheses are the number of observations on which each average is based

FUNCTION MILLI-GRAMS, PER CENT	SUBJECT	MENSTRUAL PERIOD	WEEKS FOLLOWING MENSTRUATION			
			First	Second	Third	Fourth
Urea N	E.F.	7.67 (7)	8.22 (13)	8.50 (9)	8.01 (8)	7.96 (9)
	G.H.	13.21 (7)	12.44 (11)	10.37 (9)	12.64 (9)	11.97 (7)
	Average	10.44	10.15	9.44	10.46	9.71
Total creatinine N	E.F.	1.86 (6)	1.78 (13)	1.76 (9)	1.67 (9)	1.86 (10)
	G.H.	1.95 (7)	1.86 (10)	1.93 (9)	1.87 (9)	1.80 (9)
	Average	1.91	1.81	1.85	1.77	1.83
Uric acid N	E.F.	1.15 (7)	1.15 (13)	1.12 (9)	1.12 (8)	1.06 (9)
	G.H.	1.20 (7)	1.10 (10)	1.00 (8)	1.03 (9)	0.90 (7)
	Average	1.18	1.13	1.06	1.08	0.98
Amino acid N	E.F.	9.01 (7)	8.40 (13)	8.21 (9)	7.82 (8)	8.56 (9)
	G.H.	8.72 (7)	9.36 (11)	8.76 (9)	8.94 (9)	8.66 (7)
	Average	8.86	8.86	8.49	8.41	8.60
Total non-protein nitrogen	E.F.	23.1 (7)	23.4 (13)	24.5 (9)	25.0 (8)	23.3 (9)
	G.H.	30.9 (7)	28.4 (11)	27.2 (9)	29.2 (9)	28.0 (7)
	Average	27.0	25.7	25.8	27.2	25.4
Rest nitrogen	E.F.	3.4 (7)	3.9 (13)	4.9 (9)	6.4 (8)	3.9 (9)
	G.H.	5.5 (7)	4.7 (11)	5.2 (9)	5.0 (9)	4.7 (7)
	Average	4.4	4.3	5.1	5.7	4.2
Sugar	E.F.	92 (7)	92 (13)	95 (9)	90 (8)	95 (9)
	G.H.	103 (6)	101 (11)	101 (9)	95 (8)	104 (7)
	Average	97	97	98	93	100
Cholesterol	E.F.	102 (7)	113 (12)	110 (8)	108 (8)	110 (9)
	G.H.	119 (7)	123 (10)	116 (9)	109 (9)	114 (7)
	Average	111	117	113	109	112
Inorganic phosphate	E.F.	3.23 (6)	3.36 (13)	3.35 (9)	3.45 (8)	3.21 (9)
	G.H.	3.31 (7)	3.16 (10)	3.03 (9)	3.05 (8)	2.99 (7)
	Average	3.27	3.28	3.19	3.25	3.12
Calcium	E.F.	10.19 (7)	9.95 (12)	9.98 (8)	10.00 (8)	10.41 (9)
	G.H.	10.23 (6)	10.35 (10)	9.81 (9)	10.39 (8)	10.33 (6)
	Average	10.21	10.13	9.89	10.20	10.38
Sodium chloride	E.F.	506 (6)	500 (12)	508 (8)	498 (7)	486 (7)
	G.H.	526 (6)	524 (11)	520 (9)	522 (8)	514 (6)
	Average	516	512	512	511	499
Volume per cent, corpuscles	E.F.	41.1 (6)	42.5 (13)	43.7 (9)	42.2 (8)	41.4 (9)
	G.H.	40.0 (7)	38.6 (11)	37.8 (9)	38.9 (9)	39.0 (7)
	Average	40.6	40.7	40.7	40.5	40.4

Allowing for the paucity of the data and its rather arbitrary division into weekly averages all of the above four, uric acid, total creatinine, cholesterol and chloride, may not improbably vary according to a single pattern, with the highest values occurring at about the time of menstruation and the lowest toward the close of the intermenstrual period.

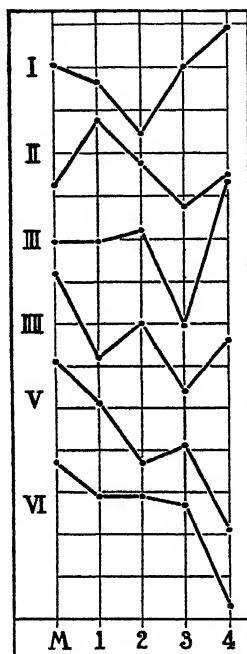


Fig. 1 Menstrual variation from the averages of table 2. M 1, 2, 3 and 4 are the menstrual period and the weeks following. I, calcium; II, cholesterol; III, sugar; IV, total creatinine; V, uric acid; VI, chloride.

Calcium is definitely lowest midway between periods.

The remaining substances, total and undetermined nitrogen, urea, amino acids, inorganic phosphate and corpuscular volume, are peculiar in that the curves for the two subjects are, in nearly every case, quite exact reciprocals of each other; so that although either subject, alone, may present a smooth curve of variation, the two are so exactly opposed that an average is unjustified and no conclusion possible.

For the sake of completeness, changes in blood-gas capacity which were described in the third report of this series (Griffith et al., '29 c) may be again referred to here. The blood oxygen and carbon dioxid capacity varied similarly, both being lowest just preceding or during menstruation and rising to a maximum during the first week following.

Discussion. That cholesterol may vary in concentration under the influence of menstruation is indicated by the finding of Guillaumin and Vignes ('28) that premature onset of menstruation is accompanied by increased cholesterolaemia and delay in menstruation by decreased cholesterol concentration. Efforts to define more precisely the cholesterol variations in normal menstruation have been made with particular thoroughness by Okey and Boyden ('27) who also review the previous work on the subject. This had been inconclusive in that according to one report blood cholesterol is highest preceding and during the menstrual period, followed by a rather sharp drop to normal; and according to another, high cholesterol values are followed by a fall immediately before menstruation, the lowest point being reached toward the end of the period, after which there is a rise followed within 2 days by a fall to the normal level which is maintained for 2 weeks. The results of Okey and Boyden, themselves, were more in harmony with the latter, consisting, as they report, of a premenstrual rise, a menstrual fall, a post-menstrual rise with a secondary fall to the normal level, the whole cycle requiring 2 weeks or more for completion. This, also, is the type of change to be observed in our data; so it would seem to be fairly established that the blood cholesterol values rise during the week following menstruation and then decline. This decline is arrested in the third week following menstruation when the concentration begins again to increase.

Cyclic variations in the blood sugar level must still be regarded as uncertain. Okey and Robb ('25) in the latest and most careful work on the subject concluded that there were none, except in the sense that the blood sugar level is more variable during the menstrual period than at other times.

This would be the conclusion, also, from the mutually contradictory results of previous work which they review. Both of our subjects are in agreement, however, in showing the lowest average values during the third week following menstruation. This is followed by a sharp pre-menstrual rise to well above the menstrual level. The only point in doubt from our data is the period immediately following menstruation. In one subject (E.F.) there is a post-menstrual rise, culminating the second week following menstruation and then dropping sharply to the low level of the third week. With the other subject (G.H.) the level is fairly constant, with only a slight fall, during the 2 weeks following menstruation and then falls sharply to the low level of the third week. Both are alike, however, it may again be repeated, in the sharp drop from the second to the third week and the equally sharp rise during the last part of the intermenstrual period to a pre-menstrual level higher than that during menstruation itself.

Although Okey and Robb failed to obtain evidence of a menstrual variation in the fasting blood sugar level, they were led to believe in a menstrual variation in carbohydrate metabolism from the observation that sugar tolerance curves resulting from the ingestion of 1.75 gm. glucose per kilo body weight showed lower maxima and a more pronounced secondary hypoglycaemia during menstruation than either just before or just after the period. This is what might be anticipated from the variation shown by our subject E.F., and the average curve in which the fasting level is higher just before and just after than during the period itself. It would be interesting to have tolerance curves for the interval at about the third week following menstruation during which both of our subjects showed the lowest fasting values.

The failure of our data to reveal any menstrual variation in blood inorganic phosphate confirms previous findings of Close and Osman ('28). Our figures, however, are rather to be described as uncertain than as positively either for or against a menstrual variation, in that each of our two subjects showed a well-defined cycle; one, however, being almost

exactly the reciprocal of the other. A similar lack of consistency characterized the findings of Okey, Stewart and Greenwood ('30) who were able only from a composite curve to arrive at a cautiously tentative suggestion that inorganic phosphate is lowest preceding menstruation and highest just after. Curiously, the average curve for our two subjects gives a quite similar result. The conclusion that such a variation is typical must wait, however, upon the collection of sufficient data to render such an average statistically sound or upon the demonstration of sufficient concurrence in individual cases.

In respect to blood calcium, Okey, Stewart and Greenwood (loc. cit.) summarize the best substantiated work preceding their own, as indicating slightly higher levels just before menstruation with a lowering during the period itself. Their own results, on the other hand, were the exact opposite of this and indicated a "tendency to frequency of low values a few days previous to the onset of menstruation and to frequency of higher values from the 8th to the 15th days following the onset of menstrual bleeding." Our own data tend rather to corroborate the earlier findings. Both subjects are quite alike in having the lowest values midway of the intermenstrual period, from which there is a rise to a maximum preceding the onset of menstruation itself.

The menstrual variation of serum chloride seems to be sufficiently clear-cut and definite to have led to complete unanimity in the preceding reports of it. Eisenhardt and Schaefer ('21), Close and Osman (loc. cit.) and Okey ('28) have all found it highest during the menstrual period; and this is in agreement with our data, in regard to which both subjects agree more closely than they do for any other of the blood constituents except uric acid and creatinine.

Non-protein nitrogen. The most thorough and painstaking study of variations of the non-protein nitrogen of the blood during menstruation is to be found in the previous work of Okey and Erikson ('26), which they have recently amplified and subjected to a very careful and critical analysis (Erikson and Okey, '31). In the first report they defined a menstrual variation of blood uric acid, which reached a high value immediately preceding menstruation amounting to 106 per cent of the intermenstrual level; this was followed during menstruation itself by a fall to a value of 86 per cent of the intermenstrual level; and was succeeded at or shortly after menstruation by a second rise to 105 per cent of the intermenstrual level. In their second report the pre- and post-menstrual rises and the menstrual depression are confirmed, but from the composite curve which they reproduce these seem to be of no more significance than other irregularities in other parts of the cycle; and the general impression, ignoring minor fluctuations, is that the values are lowest during menstruation and highest midway in the intermenstrual period. Perhaps the most significant fact, according to either of their descriptions, is that the blood uric acid level is lowest during the menstrual period itself.

This is not the evidence of our data, although we probably say so with much temerity, as opposing our meager ninety or so observations on two women to the disproportionately overwhelming number of 563 determinations on forty subjects as reported by Erikson and Okey. We merely submit as a description of our findings and await further work to reconcile or explain the difference, that both of our subjects are in unusually close agreement in showing the highest uric acid values during or just following menstruation, with a continuous fall to the lowest point just preceding the onset of the next period.

In both of their reports Okey and Erikson describe creatine, creatinine, urea and amino acids as unaffected by menstruation; this has also been reported for urea by Close and Osman (loc. cit.) and is confirmed by our data, in which the

curves for the two subjects are quite exactly the reciprocals of each other. Wang and Dentler ('20) have similarly reported no regular variation in creatine and creatinine during the cycle; their average figures for creatinine, however, are 1.35 during menstruation and 1.25 for the intermenstrual period; with, of the 15 cases studied, 9 higher during the period, 5 lower and 1 no difference; so the point might be made of a suggestion here of higher values during the period. Their conclusion of no change for creatine, however, seems the only one justified by their data; for the menstrual and intermenstrual averages are, respectively, 3.16 and 3.24 and, of the 15 subjects, 7 were lower and 8 higher during the period. So that if their figures were combined to express total creatinine, as ours do, they would indicate the possibility of higher values during menstruation; which is, also, the testimony of our subjects. Confirming Okey and Erikson, our data are negative for blood amino acids.

Finally, Okey and Erikson have found the total non-protein nitrogen of the blood decidedly elevated during the menstrual period; a rise which they find paralleled only by the undetermined nitrogen. This conclusion, again, is based on the massive evidence of 600 determinations on forty-two subjects, which makes us very glad that although our data do not entirely confirm it, neither are we required to completely disagree. The point is that our two subjects, in this respect, are quite unlike. Their curves for total non-protein nitrogen are, as might be expected, very similar to those for urea; and these have been described in the preceding paragraph as very accurate reciprocals of each other. One of them, however, is in complete agreement with the findings of Okey and Erikson, having the highest values for total non-protein and rest nitrogen (and urea) during menstruation and the lowest toward the middle of the intermenstrual period. So, although our data, alone, are inconclusive, there is partial agreement with the much better substantiated work of Okey and Erikson; and it seems justifiable to conclude that an increase in the total non-protein and undetermined nitrogen is to be expected during the menstrual period.

Okey and Erikson have made some effort to identify the component of the undetermined nitrogen responsible for its menstrual variation. The only matter of concern here is their report that it must be some element of plasma and not of the corpuscles, since these latter showed no menstrual variation in volume (hematocrit index) or hemoglobin. Our own data on corpuscle volume confirm this, again in the sense that the variations shown by each of the subjects are so exactly the reciprocal of each other that no conclusion is possible from our data. Curiously enough this is at variance with evidence provided by these same two women in regard to blood oxygen capacity (Griffith et al., '29 c) in which the menstrual is definitely lower than the intermenstrual level. It may be mentioned that this is not the only instance in our data of variance between hematocrit and oxygen capacity determinations; in connection with seasonal variation, to be referred to later, they vary inversely with respect to each other.

In conclusion, the evidence in regard to urea would seem anomalous; as has been remarked, although our two subjects do not agree with each other in the variation they show in total non-protein nitrogen or urea, these two components vary with marked similarity with each subject, there being practically a one-to-one correspondence; and this, it would seem, is what might be expected since urea constitutes such a large percentage of the total non-protein nitrogen. Indeed, in our material, the correlation between variations of urea and total non-protein nitrogen is much more exact, for each subject, than that emphasized by Okey and Erikson between the latter and undetermined nitrogen. We are not questioning the latter because it is evident in our data also; we merely raise the point that with the total non-protein nitrogen so markedly affected, as Okey and Erikson found it to be, urea would be expected to vary also and in the same sense.

Blood gas capacity. Attention has been called to our previously published finding (Griffith et al., '29 c) that the blood oxygen and carbon dioxid capacities are lowest during men-

stration and highest early in the intermenstrual period. We sincerely regret that at the time this publication was made we were unaware of previous work by Close and Osman ('28) and Okey ('28) which showed that just before or during menstruation the serum bicarbonate or the alkali reserve is lowered. On the other hand we are not sorry to have an opportunity of referring again to this matter because the variation in the closely related alveolar carbon dioxid percentage and pressure deserves the widest publicity, as being the most regular and unequivocal menstrual effect we have observed; and it would seem to be a matter of such deep-seated significance as to deserve further attention in connection with the mechanism of normal or disturbed menstruation.

In the report preceding this (Pucher et al., '34) may be found a résumé of the integrated relationships in variation that seem now to be established for the menstrual cycle: the blood-carbon dioxid capacity, carbon dioxid pressure of the alveolar air, pulmonary ventilation and acid excretion, on the one hand; and temperature, pulse and metabolic rate and nitrogen excretion on the other. These constituted two groups within each of which the variables appeared to be related according to physiological expectation. It remains to consider the nitrogen excretion in relation to the level of non-protein nitrogen; the former has been described as lowest during menstruation and rising to a maximum toward the close of the intermenstrual period; while the evidence available, as reviewed here, makes it very probable that the latter is highest during menstruation and, on the whole, follows a course the reciprocal of the nitrogen excretion. It would seem possible, therefore, to attribute the diminished excretion during menstruation to retention rather than to diminished ingestion; and the decreased metabolic rate occurring at this same time (menstrual period) to an intrinsic menstrual effect rather than to decreased protein intake. This is a conjecture, however, the merits of which can only be determined by further work.

III. SEASONAL VARIATION

During the first year of this study (1925) a blood sample was obtained from each of the four subjects, A.B., C.D. (men) and E.F., G.H. (women) approximately once a week; during the second year (1926) samples continued to be obtained from the two men at the same frequency. The variations in both years are sufficiently alike to justify combination of the data into composite curves, shown in figure 2, in which each monthly value represents the average of approximately twenty-four determinations (excepting creatinine, which was determined only during the first year and the curve of which is therefore based on approximately sixteen analyses per month). With these have been included, for the sake of completeness, the curves of blood oxygen and carbon dioxide capacity, derived from data of our third report (Griffith et al., '29 c).

The results may be summarized as indicating four types of seasonal variation:

(1) Lowest in the summer and maximum in the winter: chloride and carbon dioxide capacity.

(2) The reciprocal of the above; i.e., minimum in the winter and maximum in the summer: cholesterol, uric acid and undetermined nitrogen. Of these, cholesterol is the best established, although during the first year it showed a marked depression in the spring which would ally it with the following group. Uric acid is quite uncertain and had better be considered altogether doubtful until further data are available; it is included here only because if it has any seasonal variation it is more of this type than any other. Much the same can be said for undetermined nitrogen, although if the apparently anomalous low values for July and August are disregarded the remainder of the curve is fairly satisfying.

(3) Minimum in the spring and maximum in the late summer or fall: oxygen capacity, total non-protein nitrogen, amino acids, total creatinine, phosphate and, less certainly, urea. With the exception of urea, the evidence for this type of variation is very convincing. In our earlier report on

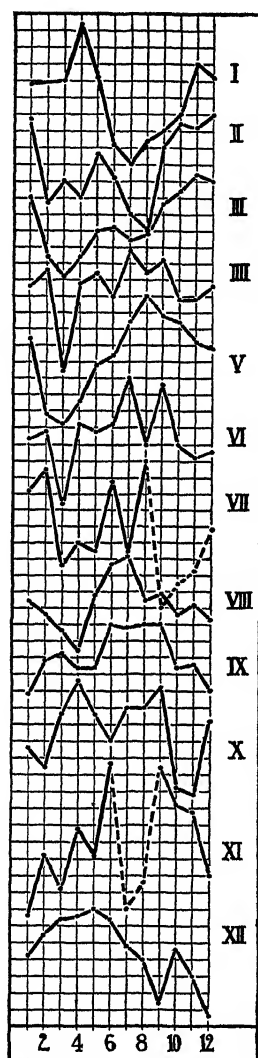


Fig. 2 Seasonal variation; 2-year averages from the data of table 3. From left to right, January to December. I, chloride; II, carbon dioxide capacity; III, oxygen capacity; IV, total non-protein N; V, amino acids; VI, total creatinine; VII, urea; VIII, inorganic phosphate; IX, cholesterol; X, uric acid; XI, undetermined N; XII, corpuscle volume.

the oxygen capacity we put more emphasis than now seems to us justifiable on the depression of the curve occurring during the summer and which would ally the variation of this function with that of the alkali reserve, as in (1) above; further consideration, however, inclines us definitely to the present classification.

(4) Finally, a single variable, the corpuscle volume, shows a type of change which is closely the reciprocal of that just described (3). In the previous section it was shown that oxygen capacity and corpuscle volume did not vary concurrently during the menstrual cycle; in that, whereas the former showed a definite variation, the latter was indeterminate; and it is but a further step from such lack of correspondence to the inverse relationship apparent here.

(5) Sugar and calcium have no recognizable seasonal variation.

It may be pointed out that types (2) and (3), above, have in common the occurrence of maxima at the same time of year; and that (4), being the reciprocal of (3), these might, not illogically all be regarded as effects of a common cause and, to that extent, fundamentally one type of change.

Discussion. We know of no previous literature on seasonal variation of blood constituents, except two reports on phosphate. Hess and Lundagen ('22) have described a variation of the blood of infants similar to that shown by our data; according to them, however, this was much less marked in older children and lacking in adults. We are confirmed in our result, however, by Havard and Reay ('25) who also obtained a variation in adults but with the minimum in January. It is not improbable, however, that the maxima and minima must depend on the conditions affecting the group studied; and the important point would seem to be the general agreement that the blood phosphate level is subject to seasonal change.

In conclusion, it may not be inappropriate in this last number of this series of reports to summarize the evidence which our data seem to reveal of seasonal variability in physiological activity. The backbone of this evidence is provided by

two functions which not only afford unequivocal testimony of seasonal variability, but which were capable of such direct measurement as to make it altogether improbable that the result is an artefact of analytical error; these are the basal pulse rate (Griffith et al., '29 b) and the volume of pulmonary ventilation under basal conditions (*ibid.*, '29 d). According to the averages for our group, the first was lowest in August and highest in February; the second, lowest in April and highest in August-September. It will be noted at once and will be returned to later that, considering the period February-April as a unit, these are reciprocals of each other.

These two functions are regarded as fundamental, not in the sense of being considered the cause of any other variation that may appear, but rather as furnishing indubitable evidence that variation of two distinct types does occur. For, with respect to these two functions, all of the subjects of our group behaved alike during both of the years of our study. But the inference would seem sound that variation in these would be due to or accompanied by variation in other physiological processes.

Conclusions regarding other functions which give any evidence of seasonal variation are less certain than for the two just mentioned in that the individual curves are less regular and there is less exact correspondence between the maxima and minima of the curves for the different subjects; these are irregularities that might result from less direct and precise methods of measurement; or from the fact that the functions, themselves, are subject to subsidiary influences of unequal incidence with the various members of the group.

However, in practically all cases where there is sufficient concurrence among enough members of the group to give trustworthy evidence of seasonal variation in the average curve, this is found to belong to one or the other of the following two major patterns.

I

1. Basal pulse rate.
2. Body weight.
3. Basal metabolic rate; oxygen consumption per minute and calories per square meter per hour.
4. Basal excretion of water, chloride and nitrogen (excepting ammonia).
5. Blood chloride and carbon dioxid capacity.

II

1. Respiratory minute and tidal volumes with correlated variation in rate and the composition of the expired air.
2. Blood (a) oxygen capacity (and inversely corpuscle volume) (b) phosphate and cholesterol (c) nitrogen.
3. Basal excretion of total and organic acid and ammonia.
4. Oral temperature.
5. Basal and standing systolic blood pressure.

In spite of some scattering of the maxima and minima the first group may be said to represent a type of change in which the low values occur quite uniformly in August with high values in the period, February–March; in the second the high values are more evenly distributed over the period, August–October, and the low from March to May. It is not improbable that further work may necessitate some revision of this classification; or, granting indubitable seasonal changes and the general validity of this grouping, it is not unlikely that individual members of each of these groups may have really distinct maxima and minima and degrees of precedence as the seasons change.

However, for the moment and for the support thereby provided toward the establishment of these as bona fide physiological, rather than fortuitous variations, we are inclined to minimize the individual differences, as being due, perhaps in large measure, to insufficient data, and to emphasize the striking evidences of interrelationship which then become evident.

It is not possible to account for all the relationships within each group, but for the most part they are entirely rational. Thus, without any preconceived bias for or against seasonal variation in metabolism, the fact that weight, basal pulse and metabolic rate and nitrogen excretion all vary concurrently would seem strong proof that no one of them is merely accidental and fortuitous; likewise, if the blood chloride shows any variation it is not unexpected that chloride and water excretion would change similarly. Within the second group, the correlated variations of the respiratory functions have been commented upon previously (Griffith et al., '29 d) and need not be gone into in detail here; the variation of pulmonary ventilation appears adapted, also, to changes in acid-base equilibrium as shown by excretion of total and organic acid and ammonia; and, finally, most of the blood constituents vary similarly.

Likewise, conviction that we are dealing with a unified, organic response to changing conditions throughout the year receives support from the interrelationships between certain important members of each of the two groups. Thus, the variation in basal nitrogen excretion is evidently not due to changes in intake (Pucher et al., '34) so the evidence of an inverse relation to the blood nitrogen level provides the only other satisfactory explanation for the changes in both; and, finally, the alkali reserve is lowest just when the pulmonary ventilation and acid excretion are highest and vice versa.

From considerations such as these we feel confident, therefore, that a general, uniform variation in physiological activity is an established possibility; and this is all that we have been concerned to prove. Our opinion, the reasons for which were developed in the first paper of this series, that such variations as these are very probably not due to the operation of any one factor but express the integrated effect of many, would seem to have the virtue of reconciling such diverse results as have already been reported and to emphasize the need for extreme caution in generalizing from any one set of data. If, however, the possibility of cyclical variation is

accepted it follows that differences of the magnitudes involved must be allowed for in long-continued studies or in comparison of results obtained at different times; and interest is stimulated in the search for the causative factors that may be involved.

SUMMARY

This is the sixth and last of a series of reports dealing with intra-individual variation in a number of simultaneously determined physiological functions.

1. Statistics are given of the maximum and minimum, mode, mean, standard deviation and coefficient of variation for each of the following blood constituents: total non-protein, urea, amino acid, uric acid, total creatinine and undetermined nitrogen; sugar, cholesterol, inorganic phosphate, calcium, chloride and corpuscle volume.

2. Comparison of the coefficients of variation for the blood constituents and their urinary homologues (preceding paper) provides a statistical measure of the greater stability of the blood composition.

3. There is no correlation between the day-to-day, intra-individual variations of any of the blood constituents studied and similar variations in the basal metabolic rate.

4. There is evidence of menstrual variation as follows:

Cholesterol: rises during the week following menstruation; falls during the two following weeks; then begins to rise preceding the onset of the next menstrual period. Sugar and inorganic phosphate are doubtful but perhaps similar to cholesterol.

Calcium: highest during or just preceding menstruation and lowest midway of the intermenstrual period.

Chloride, uric acid and total creatinine: these show the most clear-cut cycles of any of the blood constituents, according to our data, and are highest during menstruation and lowest toward the end of the intermenstrual period.

Total non-protein, urea, amino acid and undetermined nitrogen: our data are doubtful but are in partial agreement with very trustworthy previous evidence for a cycle very similar to that for chloride, uric acid and total creatinine, above.

Blood-gas capacity (oxygen and carbon dioxid; from data in our third report) is lowest during menstruation and highest in the early part of the intermenstrual period.

5. There is evidence for seasonal variation as follows:

Chloride is lowest in the summer and highest in the winter; the same is true of the blood-carbon dioxid capacity (data from our third report).

Cholesterol, inorganic phosphate, amino acid, total creatinine, total non-protein and possibly, though less certainly, urea and undetermined nitrogen are highest during the summer or fall (July–September). For cholesterol and undetermined nitrogen the minima are in the winter (January); for the others it is during the spring (March–April). A similar variation is shown by the blood oxygen capacity (third report), while the corpuscle volume varies reciprocally, being highest in the spring and lowest in the fall.

Uric acid is very doubtful and calcium and sugar certainly give no evidence of seasonal variation.

6. By way of summary, attention is called to the evidence from our data of two major types of seasonal variation, roughly the reciprocal of each other: 1) Maximum in the winter and minimum in the summer; blood chloride and carbon dioxid capacity, urine volume, chloride and nitrogen excretion under basal conditions; weight and basal pulse and metabolic rate; 2) maximum in the summer or fall and minimum in the winter or (usually) spring: most of the blood constituents, as above; respiratory activity and urinary acid excretion under basal conditions.

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THE NUTRITIVE VALUE OF ANIMAL TISSUES IN GROWTH, REPRODUCTION AND LACTATION ¹

I. ALCOHOL-EXTRACTED BEEF LIVER

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INTRODUCTION

In most studies of growth and reproduction on meat diets, whole, raw, cooked, or cooked and dried tissues have been used. The biological differences shown to exist between the various tissues obviously reside not only in the protein, but also in the quality and quantity of other constituents. In order further to segregate these factors a series of experiments was planned in which the tissues that formed the source of protein were subjected to prolonged extraction with alcohol until fat-free. By the use of such materials the protein factor should more nearly be reduced to a single variable and any other important dietary contribution made by the tissue as a whole could be more clearly revealed. Beef liver was used in the present work and experiments with other tissues are in progress.

As early as 1917, Osborne and Mendel ('17) demonstrated improved growth resulting from small supplements of liver

¹ This research was supported in part by the National Live Stock and Meat Board through the committee on grants of the National Research Council. Progress reports have been presented before the Iowa branch of the Society for Experimental Biology and Medicine, Proc. Soc. Exp. Biol. and Med., 1931, vol. 28, p. 597; 1932, vol. 29, p. 669; 1932, vol. 30, p. 365.

² Doctor Smith had completed the notes for this paper a few days before his death. The data were somewhat reorganized with the assistance of Dr. H. A. Mattill. W.H.S.

to a lean beef diet, and their later experiments ('26) indicated that liver supplied the growth accelerating factors now commonly known as vitamins A and B. The extensive experiments of McCollum ('21) and his co-workers on the nutritive properties of animal tissues showed that 20 per cent of whole liver in the diet suffices as the sole source of protein for normal growth, reproduction, and rearing of young. Since the earlier work has been extensively reviewed in recent reports, especially by Clayton ('30), Daggs ('31) and Graham and Griffith ('27), detailed presentation is unnecessary at this time. Clayton, in her comparative study, investigated the relative efficiency of beef round, liver and kidney in both cooked and raw forms as sources of proteins and accessories, primarily for growth and reproduction. Qualitative and quantitative variations in the fat, as well as variations in the vitamin content of her diets, made the interpretation of the results as to the relative value of the proteins, as such, rather difficult, although kidney protein appeared to be superior, liver ranking next and muscle last.

EXPERIMENTAL

Animals. Albino rats obtained from the Sprague-Dawley Company, Madison, Wisconsin, were placed on experiment when they weighed 35 to 40 gm. They were kept in individual, wire-bottomed cages which were cleaned and sterilized at frequent intervals. Food cups were weighed and filled frequently so that the animals had access to food at all times. The animals were weighed every 5 days. The original lot was started in the first week of March, 1931, and later lots were started at approximately the same time of the year, in order to rule out seasonal variations between series, in so far as possible. Individual records, containing the growth curves, food consumption and breeding history of each animal, were carefully kept.

Rations. The rations were constituted as follows:

RATION	EXTRACTED LIVER	FAT	STARCH	PROTEIN PER CENT	ASH PER CENT	NUTRITIVE RATIO
A	18.7	15.0	60.3	15.0	4.3	1: 6.5
B	24.6	15.0	54.4	20.0	4.4	1: 4.6

Both rations: Agar, 2 per cent; salt mixture,¹ 4 per cent; cal. per gram, 4.6 (calc.).

¹ Osborne and Mendel salt mixture.

Hydrogenated cottonseed oil³ was used to supply the fat of the rations, because it does not contain sufficient vitamin A to supply the needs of white rats, but does contain adequate amounts of vitamin E. Moreover, material of uniform quality could always be obtained.

The fat-free liver, prepared by extracting beef liver, fresh from the slaughter house, with hot alcohol (Bloor, '26, p. 36) for 50 to 60 hours, had a protein content ($N \times 6.25$) of 85 per cent and ash, 1.7 per cent. For calculating the composition of the diets, the remainder (13.3 per cent) was considered to be carbohydrate.

Preliminary experiments demonstrated that the diets were very deficient in vitamins A and B,⁴ and these factors were supplied by the daily administration of 0.5 gm. of dried yeast⁵ and 0.5 cc. of vitamin-tested cod-liver oil.

Growth. Growth records were kept for 120 days, although comparisons were finally made for the first 90 days only. Growth on the 20 per cent protein level (ration B) was normal or slightly above, whereas on the 15 per cent level (ration A) it was below normal (table 1). With few exceptions, the animals remained well and active for 250 to 275 days, although

³ Crisco.

⁴ In a paper on the nutritive value of liver, McHargue and co-workers ('30) present apparently quite different results. Their alcohol extraction of raw liver, which they do not describe, was doubtless less complete than ours, and their manner of preparing whole dried liver probably made it of as little value, so far as vitamins A and B are concerned, as our alcohol extracted liver.

⁵ Kindly furnished by the Northwestern Yeast Company.

on autopsy it was not uncommon to find badly diseased lungs. Abnormally appearing organs other than this were rare.

Reproduction. All the females on the rations were fertile. The first matings were not controlled by vaginal smears, but these were begun when the animals were approximately 180 days old and after most of them had produced one or two litters. The estrous cycles at that time were irregular and marked by abnormally long periods of continued cornification and especially of resting stages. Later studies showed this to be the usual condition on the extracted liver diets. There was considerable difficulty in obtaining positive matings, in

TABLE 1

Gains in weight during 90-day period from weaning on extracted-liver rations

BASAL RATION	SEX	YEAST AND COD-LIVER OIL		YEAST, COD-LIVER OIL AND DRIED WHOLE LIVER (0.5 GM.)	
		1st generation ¹	2nd generation ¹	1st generation	2nd generation
		Gm.	Gm.	Gm.	Gm.
A	♂	186(8) ²	162(7)	242(4)	231(4)
A	♀	148(10)	134(9)	171(4)	150(4)
B	♂	225(7)	199(6)	258(4)	277(4)
B	♀	162(8)	169(7)	172(4)	158(4)

¹Includes series 1 (1931) and 2 (1932).

²Number of animals.

part due to incipient sterility in the males, but not entirely so, since the difficulty continued when vigorous stock males were used for matings. The testes of the experimental animals were normal in weight and the epididymes contained abundant, although very sluggish, sperm. Once positive matings were obtained, normal litters were cast. The abnormal cycles and difficulty in mating led to considerable loss of time, prevented an adequate reproduction history of the animals and limited the size of the experimental groups.

Lactation. Litters were reduced to six young within the first day or two after birth and weaned whenever possible, at 21 days of age. Lactation on the extracted liver diets was not entirely successful (table 2). In series 1 (1931), 54 and

51 per cent of the young born on rations A and B, respectively, were weaned. In series 2 (1932), carried through exactly a year later, only 23 and 22 per cent, respectively, of

TABLE 2
Lactation on extracted liver. First generation

RATION	SERIES	FEMALES	TOTAL LITTERS	TOTAL YOUNG	YOUNG WEANED	
					Number	Per cent ¹
A	1	3	9	59	30	54
A	2	3	9	69	14	23
					Av.	38.5
B	1	3	9	70	34	51
B	2	2	4	28	6	22
					Av.	36.5
A	Daily supplement of	3	9	71	29	48.3
B	0.5 gm. dried whole liver	3	9	75	23	30.7

¹ Weaning percentage calculated from the number of animals left after reducing size of litters.

TABLE 3
Lactation on extracted liver. First generation. Number and weight of young at weaning (21 days)

RATION	SERIES	FIRST LITTERS		SECOND LITTERS		THIRD LITTERS	
		Number of young weaned	Average weight	Number of young weaned	Average weight	Number of young weaned	Average weight
A	1	17	Gm. 25.5	10	Gm. 14.0	3	11.3
A	2	12	17.0	2	21.0	—	..
B	1	16	28.3	10	20.9	8	16
B	2	6	18.0	—	..
A	Daily supplement of	13	22.5	11	21.4	5	20
B	0.5 gm. dried whole liver	17	30.3	6	24.3	—	..

the young were weaned. First litter animals of series 1 were normal at 21 days, those of series 2 and the later litters of series 1 were definitely below normal weight (table 3). Although the weaning percentage was no greater on ration B

than on A, the higher protein level led to a better growth of the nursing young, suggesting better milk production. The difference in weaning between the two series, although unexplainable, is not a new experience. Clayton, for example, had weaning percentages of 39 and 8 in two lots of animals on her liver ration 130. Variation in the original animals undoubtedly is a factor and can only be controlled by the use of a larger number of animals from the same stock. The females usually lost weight during the lactation period; the losses averaged as much as 4 gm. daily and were generally less on the 20 per cent protein diet. It is impossible to state whether inability to nurse the young was due to scanty milk production, poor quality of milk or both. In most cases the milk band was apparent for the first day or two, but usually less distinct than one would expect. In many cases there were no visible signs of lactation.

Experiments to be reported later indicate that inadequate lactation was not due to a lack of any of the known vitamins, especially the vitamin B complex, which was furnished at a uniform, but perhaps minimal level to all animals recorded.

Second generation

Some of the second generation animals were continued on the diets supplemented with yeast and cod-liver oil. The growth of these animals (table 1) was inferior to that of the first generation, except for the females on ration B, whose growth rate approximated that of the females on the same diet in the first generation. The weaning weights of these particular animals were exceptionally high, which may account for their subsequent better growth. Since the young had a slow and varied growth rate, it was not possible to set an arbitrary weight or age for their removal. When they appeared to be able to eat for themselves, the most vigorous ones were placed on the experimental diets, usually at about 30 days of age. Their subsequent inferior growth rate as compared with that of the parent generation was probably due to the fact that their parents, the offspring of stock

colony rats, had been more heavily endowed during early life with certain necessary nutritive factors.

Reproduction was subnormal in the second generation. Estrous cycles were more irregular than in the first generation and it was even more difficult to obtain positive matings. At the end of a long period, some of the animals had borne only two or three litters and were in such poor physical condition that breeding experiments were discontinued. The males were almost entirely sterile, as shown by matings with normal females at estrus; testis weights were normal, but live sperm were seldom found in the epididymis.

Lactation was a failure in this generation. Young of nearly normal size were born, but most of them died within the first few days. In all, 26 litters, containing approximately 180 young, were born, and only 10 were weaned, with an average weight of 17 gm. at 21 days.

After certain animals had twice failed to rear their young, an attempt was made to restore normal lactation by a supplement of raw liver (1.5 gm. per day, equivalent to 0.45 gm. dry weight) beginning usually at the time of the placental blood-leak. In over half of these instances young were weaned, with weaning weights ranging from 25 to 40 gm. at 21 days. Apparently, raw liver furnished some factor, necessary for lactation, in which the basal rations were deficient, and which had not been supplied by yeast and cod-liver oil or by Harris vitamin concentrate or wheat germ oil of proved potency. Wilkinson and Nelson ('31) have reported that the addition of raw beef or hog liver to deficient diets decreased the mortality and increased the weight of the nursing young. Daggs ('31) showed that dogs on liver diets produced milk with a greater fat content than did those on diets containing other sources of protein; puppies nursed by mothers on liver diets grew faster. He suggests that liver is a better food for milk production, because "it may contain some hormone or lactation-producing vitamin in greater abundance. . . ." More recently, Mapson ('32) has demonstrated a favorable influence of raw liver upon growth and lactation in rats.

The occurrence in sources other than liver of unidentified substances favoring growth and lactation and which do not seem to be related to the known vitamins has also been suggested by Mason ('21) and Evans and Burr ('27) in lettuce, Coward ('29, '31) in casein and Sherman and his co-workers ('22, '24) in milk. Future developments in the study of the vitamin B complex may relate these substances to one or another of the B fractions, but this seems doubtful at the present time.

Simultaneously with series 2 (1932), a group of young animals was placed on the basal rations A and B supplemented with yeast and cod-liver oil, and also with $\frac{1}{2}$ gm. of dried whole liver⁶ daily. As is shown in table 1, these animals made considerably better gains than those not receiving whole liver. The most marked improvement was with the ration A animals. As judged by regularity of estrus, per cent weaning and weaning weights of the young (tables 2 and 3) the reproductive performance of these animals was considerably better than that of the simultaneous series 2 animals. Some of the young were continued on the same dietary (table 1, last column). The second generation male animals made more rapid gains than the females; later work has shown this to be the usual result of whole liver feeding. The animals were fertile, but lactation was not a success. Eleven litters were born to eight females but only three succeeded in rearing young, and these experiments were discontinued. Since dried whole liver was not as effective as raw liver in supporting lactation it was at first presumed that the lactation factor was different from the growth-promoting factor. Later experiments indicated that dried whole liver also aids lactation and that the difference is quantitative rather than qualitative. This is in line with the observations of Wilkinson and Nelson ('31) that the galactagog factor in liver is destroyed by drying the tissue at 120°C.

⁶ Dried in a Bufllovak, steam-heated, vacuum oven at 70 to 100°C.

Third generation

The animals of this generation were only those suckled by mothers that received the raw liver supplement and they were continued on the original basal rations, unsupplemented except for yeast and cod-liver oil. Owing to the impossibility of maintaining an adequate supply of alcohol-extracted liver about twenty animals having a similar growth-rate were discarded after 60 days on the rations. Table 4 therefore contains figures obtained both at 60 and at 90 days and shows that growth in the third generation was decidedly superior

TABLE 4
Growth on extracted-liver rations. Third generation

RATION	SEX	AVERAGE GAIN IN WEIGHT (GM.)	
		60 days	90 days
A	♂	172(11) ¹	212(2)
A	♀	124(9)	153(4)
B	♂	226(5)	249(2)
B	♀	142(3)	171(3)

¹ Number of animals.

to that of the second generation and slightly better than that of the first. From observations comparable to these, Mapson assumed the transmission of a growth-promoting factor. This was reflected in the breeding history of this group. The males were all fertile and the females showed less variation in estrous cycles, although positive matings were again hard to obtain. Normal offspring were produced and lactation was partially successful. In all nineteen litters were obtained and twenty-six young were weaned. This third generation, whose parents received supplements of raw liver, weaned 21 per cent of their young (based on elimination to six), as compared with 37 per cent for the first generation and 0 in the second before the addition of raw liver.

Fourth generation

Growth in this generation was comparable to that in the third. The males were fertile and normal litters were born. The animals were followed through two litters each and in all cases there was a complete failure of lactation. The effectiveness of the raw liver supplement, given the second generation animals, in improving the nutritional status of the third generation animals apparently extended in a small part to the fourth generation.

DISCUSSION

It is unlikely that the improved growth and reproduction following whole liver administration was the result of an increased intake of the vitamin B complex as this is ordinarily accepted. Since the protein in these rations was to be limited to that furnished by the extracted-liver, when additional amounts of the B complex were to be provided, the Harris vitamin concentrate was used. When administered in amounts six to ten times what is generally considered adequate this concentrate failed to restore lactation while 1.5 gm. of raw liver was strikingly successful.

Nor is it probable that the protein of the whole liver supplements was the effective factor since the feeding of whole liver produced an increased growth rate in animals on both rations. While those on the 15 per cent protein ration showed the more striking response, the consumption of 0.5 gm. dried whole liver or its equivalent daily, by each animal, increased the protein intake only to that of the animals on the 20 per cent ration. Furthermore it was determined by a limited number of experiments that the feeding of an equivalent amount of extracted liver to animals on ration B produced a negligible improvement.

Slonaker ('31) studied reproduction on various levels of protein and found a considerably higher weaning percentage, just as he did better growth, on a 14 per cent level of protein than on a higher level. He found a decrease in the size of the litters as the protein was increased, accompanied by a heavier

average weight of the young at birth. His basal diet contained 10 per cent protein distributed approximately equally between plant and animal sources and the higher percentages were obtained by adding meat scrap; the quality of protein was thus not uniform in all his rations. On our extracted liver diets in the first generation there were no significant differences in the weaning percentages on the two levels (table 2), or in the average number of young born per litter or in the average weight at birth (table 3). Supplementing the diets of the second generation with raw liver appears to have increased the average number per litter as well as the

TABLE 5

Influence of protein in the diet on number and weight of young at birth

Generation	1		2 ¹		3		4	
Ration	A	B	A	B	A	B	A	B
Av. number per litter	7.0	7.5	6.7	5.7	8.5 ²	6.7 ²	7.1	10.0
Av. weight (gm.)	5.3	5.2	4.9	5.0	5.4	5.6	5.8	5.2
Ration, plus raw liver supplement								
Av. number			8.8	6.0	7.3	9.3		
Av. weight			5.5	5.5	5.2	5.8		

¹ Offspring of series 1.

² The mothers of these animals received 1.5 gm. of raw liver from the twelfth day of pregnancy through lactation.

average weight (table 5). The figures for the succeeding generations are variable and are less significant because of the smaller number of animals involved.

The vitamin E requirement was furnished by the 15 per cent of hydrogenated cottonseed oil in the rations. During the course of this work over 150 litters were born and there were no resorptions. The lactation deficiency was not a manifestation of a lack of E since an excessive amount of wheat germ oil was ineffective as a cure.

The irregular estrous cycles in the females and the impotence in the males can be correlated with the poor physical condition of the animals. The improved nutritive condition of the third and fourth generations was reflected in a greater

regularity of estrus in the females and increased fertility in the males. Evans and Bishop ('22) showed that quantitative undernutrition, produced by limited food intake, seriously interfered with sexual maturity and ovulation. Guilbert and Goss ('32) have presented a reproductive history of rats maintained on low protein (3.5 to 5.0 per cent) diets similar to that obtained on the extracted liver rations, a) irregular cycles, b) repeated failure of the females to mate at estrus when placed with normal males, and c) apparently infertile matings with recurrence of estrus. Irregular ovulation on heat-treated or alcohol-extracted casein diets has also been reported by Coward ('29, '31).

The removal of essential nutritive factors from animal foods by hot alcohol extraction appears to be an effective approach to the problem of identifying the tissue constituents with known or as yet unknown dietary requisites. The feeding of protein in such extracted form gives a more nearly true measure of its nutritive value. A study of other animal tissues is in progress.

SUMMARY

When alcohol-extracted liver furnished the protein of synthetic rations, all other recognized dietary requisites being supplied in uniform and presumably adequate amounts, growth of rats was normal at a protein level of 20 per cent; on a 15 per cent level growth was distinctly subnormal. In the second generation growth was below normal on both levels of protein. Lactation was deficient in the first generation and a failure in the second generation.

Supplementary feeding of dried whole liver increased the growth rate of first and second generation animals; raw liver supplements enabled the females to lactate and led to the weaning of more vigorous young, to better growth in the two succeeding generations, and to partial success in lactation in the third generation.

The reproductive mechanism was seriously deranged on these alcohol-extracted liver rations, as evidenced by irregular cycles and failure to mate at estrus.

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THE NUTRITIVE VALUE OF ANIMAL TISSUES IN GROWTH, REPRODUCTION AND LACTATION

II. THE PRESENCE OF A NEW DIETARY PRINCIPLE IN LIVER¹

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TWO FIGURES

In the previous paper (Smith and Seegers, '34) it was shown that dried whole beef liver possessed a growth-promoting factor which alcohol-extracted liver had lost; this factor did not appear to be protein nor was it identified with any of the known vitamins. The reproductive capacity of rats on extracted liver declined in the first generation and almost disappeared in the second. Especially striking was the improved lactation secured by small supplements of raw liver added to a diet of extracted liver, and the advantage thereby conferred on the young (which did not themselves receive raw liver) was manifested by their better record of growth, reproduction and lactation as compared with that of their parents before these were given the raw liver supplement. These observations required confirmation and amplification, particularly as to the chemical nature of the growth and lactation factors and their possible identity with each other or with other already recognized food accessories.

¹ This research was supported in part by the National Live Stock and Meat Board through the committee on grants of the National Research Council.

² Deceased.

³ The experimental work reported in this paper was directed by Dr. H. Gregg Smith. The manuscript was prepared with the assistance of Dr. H. A. Mattill.

W. H. S.

The work described in this paper was designed first of all to demonstrate that the superiority of raw liver and of dried whole liver over extracted liver was real and not a fortuitous result. In order to reveal this advantage more definitely dried whole liver and alcohol-extracted liver were used, in the first series of experiments, not as small supplements but as the sole source of protein in otherwise identical diets.

Rations. With materials prepared as previously described and by the same experimental procedure (Smith and Seegers, '34) ration A containing 15 per cent protein in the form of alcohol-extracted liver was compared with a similar diet (ration C) containing the same amount of protein in the form

TABLE 1

	<i>Ration A</i> <i>Per cent</i>	<i>Ration C</i> <i>Per cent</i>
Alcohol-extracted liver	18.7	
Dried whole liver ¹		23.2
Hydrogenated cottonseed oil ²	15.0	10.7
Cornstarch	59.8	61.6
Salts ³	4.5	4.5
Agar agar	2.0	2.0

¹ Heated in a Bufllovak, steam-heated vacuum oven at 70 to 100°C.

² Crisco.

³ Osborne and Mendel as modified by Hawk and Oser ('31).

of dried whole liver. The composition of these rations is given in table 1. Except where otherwise stated, they were supplemented daily with 0.5 gm. of yeast⁴ and 0.5 cc. of cod-liver oil.

Growth. Chart 1 shows the average gain in weight of six female and three male animals on each of the two rations (series 6 and 7), and of a like number on ration A supplemented with 0.5 gm. raw liver (series 8). Differences in growth response on whole liver were immediately noticeable; whether this was provided as dried whole liver (diet C) or as a supplement of raw liver to the extracted liver (diet A), growth was accelerated, particularly in the male animals. The total capacity for growth was also increased; the animals

⁴ The yeast was kindly furnished by the Northwestern Yeast Company.

were heavier and more vigorous throughout life. After about 50 days those on the raw liver supplement began to lag behind those on the dried whole liver. This difference, which also continued throughout life, leads us to conclude, contrary to

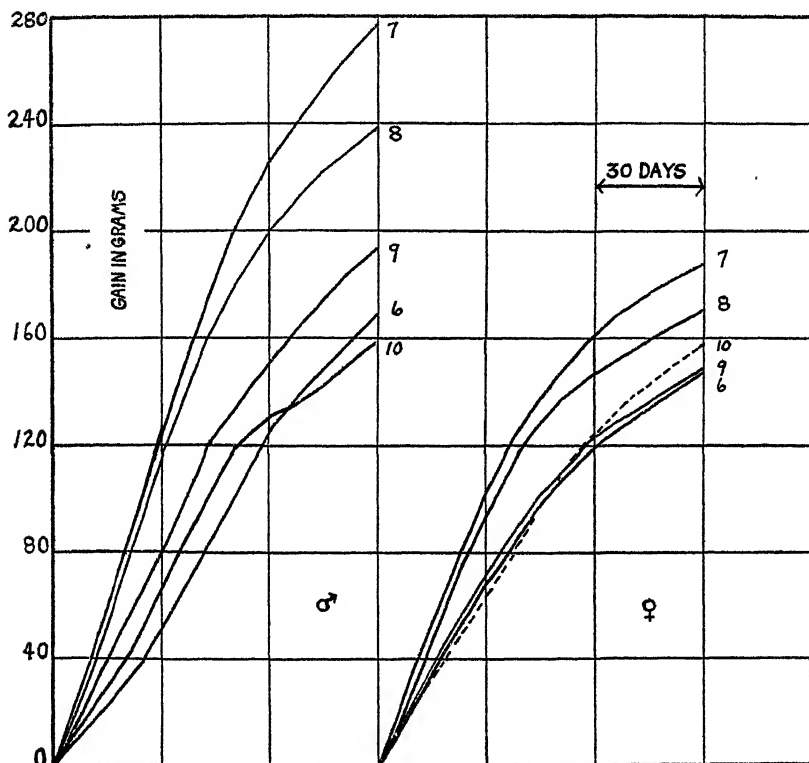


Chart 1 The curves represent the gains made by rats on the dried whole (7) and extracted-liver (6) rations, and the gains made when the extracted-liver ration was supplemented with raw liver (8), Harris vitamin concentrate (9), and a water soluble fraction of liver (10).

the statements of Mapson ('32) that the maximum effect of this particular growth factor in liver is not produced by that amount of it which is contained in 0.5 gm. raw liver. More than 0.5 gm. is necessary for optimum growth.

Food consumption. Records of food consumption have been kept for these series as well as for some 300 other animals

in the course of these studies. The amount of food ingested per gram increase in body weight has always been less when whole liver is fed. Figures for these three series and two others are given in table 2, and they clearly indicate a greater economy in the use of food and of its protein when whole liver is in the diet. The relation between these figures and the growth curves is interesting; the rapidly growing animals put more food into new tissue and less into maintenance than the more slowly growing. A note with special reference to

TABLE 2
Food consumed during 90-day growth period

DIET AND SUPPLEMENT	SERIES	AVERAGE GAIN IN WEIGHT		TOTAL FOOD PER GRAM GAIN IN WEIGHT		PROTEIN PER GRAM GAIN IN WEIGHT	
		♂	♀	♂	♀	♂	♀
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
Diet A (extracted liver)	6	174	152	5.4	6.0	0.81	0.90
Diet C (dried whole liver)	7	278	189	3.8	5.2	0.57	0.77
Diet A + raw liver (0.5 gm.)	8	239	175	4.7 ¹	5.4	0.79 ¹	0.92
Diet A + Harris conc. (300 mg.)	9	187	152	5.9	6.1	0.80	0.86
Diet A + water fraction	10	158	162	5.9	5.9	0.81	0.85

¹ Including the raw liver supplement.

food intake and its relation to growth and efficiency quotients with paired feeding experiments was recently published ('32). While some workers have interpreted the growth-promoting effect of liver as a stimulation of the appetite we are inclined to favor the view expressed by Rose in the general statement ('28) that "when an added dietary component leads to an appetite stimulation the explanation is to be found, we believe, in the influence exerted upon the cells themselves."

Reproduction and lactation (table 3). In the breeding experiments females were mated with males on the same dietary. In series 6 (extracted liver) the estrous cycles were again

irregular and were marked by abnormally long periods of cornification and resting stages, with the latter predominating. With one exception after two litters were obtained from each animal on ration A reproduction was discontinued; from our experience the subsequent reproductive performance on this diet would have been unsatisfactory. Reproduction declined much more slowly in series 7 and 8 which received whole liver.

TABLE 3
Summary of reproduction

DIET AND SUPPLEMENT	SERIES	NUMBER OF FEMALES	NUMBER OF YOUNG BORN AND THEIR AVERAGE WEIGHT						PER CENT WEANED ¹	AVERAGE WEIGHT AT 21 DAYS
			1st litters		2nd litters		3rd litters			
			Number	Weight	Number	Weight	Number	Weight		
Diet A (extracted liver)	6	6	40	5.0	31	4.9	5	5.8	0	0
Diet C (dried whole liver)	7	5	51	5.7	38	5.7	22	5.7 ²	26.9	38.4
Diet A + raw liver (0.5 gm.)	8	6	43	5.8	44	5.3	26	5.4	34.0	19.5
Diet A + Harris conc. (300 mg.)	9	6	41	5.5	16	5.1	18	5.6	3.8	15.7
Diet A + water fraction	10	6	45	5.4	33	5.0	30	5.4	0	0

¹ Based on elimination to six, and is an average from all litters.

² Based on incomplete data.

From outward appearances all the young were normal at birth but their weight and number appear to have been influenced by the diet. The average number of young per litter in series 7 on dried whole liver was 8.4 with an average weight of 5.7 gm. per animal. Corresponding figures on the extracted-liver diet, series 6, were 6.3 and 5.1 gm. The results with the raw liver supplement, series 8, compare favorably with those of series 7. The precise reasons for the variations in the number and the weight of the young can only be determined by more extensive experiments. The number of young was doubtless influenced by the nutritional condition of the mother. Their weight may have been increased by the mother's immediate ability to lactate before the young were

weighed for the first time, and without specially devised cages it is impossible to determine the birth weight.

In comparison with earlier observations (Smith and Seegers, '34) the mortality of the young in these series was higher than expected, but such fluctuations are not uncommon and have also been referred to. Differences between series have at least been consistent. Lactation was a complete failure on the extracted-liver ration A. Both raw and dried whole liver enabled the females to nurse some of their young. Their weight at the time of weaning (21 days) was high compared to that of the young in the first series on ration A (Smith and Seegers, '34, table 3), where the protocols show a progressive decline in average weaning weights to as low as 11 gm. The marked difference in the weight of the young at the time of weaning clearly indicates that the growth response to liver feeding takes place early in life; and although the evidence is incomplete for reasons already stated, the response may even take place in uterine life. This is in part contrary to the observations of Mapson ('32) who believes that the time at which the growth response manifests itself in the liver-bred offspring is approximately that at which they begin eating for themselves. The inferior weight of the young, which mothers on the extracted-liver rations succeed in rearing, is a characteristic of the absence of the growth factor. The mothers lost weight during the lactation period, but this loss was no more than could be expected on a 15 per cent protein level (Kozłowska, McCay and Maynard, '32).

The difference in the nutritive value of the extracted and whole liver shows conclusively that alcohol extraction removes material from liver which is necessary for optimum growth and lactation. Since both diets (A and C) contained liver protein in equal quantities, it can be concluded that liver protein is not the influencing factor. This conclusion rests on the assumption that the extracted liver protein was not damaged in the process of preparing it. The validity of this assumption has experimental support in the showing made by series 8.

The lipid constituents of liver do not contain the active substance. Liver fat (ether soluble portion of alcohol extract) was substituted for the hydrogenated cottonseed oil in ration A to the extent of 6 per cent of the diet. This quantity of liver fat is approximately equivalent to the amount present in diet C. The growth of six animals was compared with that of six further controls on ration A. At the end of 60 days there was no difference between them and the experiment was terminated. That liver fat does not aid lactation is indicated by the fact that there was no improvement in lactation when liver fat (equivalent to 1.5 gm. raw liver) was given to female rats receiving basal ration A. This is in agreement with the conclusions of Wilkinson and Nelson ('31).

The possibility of the identity of the growth-promoting factor with the thermostable factors in yeast was given particular consideration. In the first of these experiments (series 9) 300 mg. of a yeast concentrate (Harris) was fed daily in addition to the 0.5 gm. of dried yeast which was the minimum daily allotment for all animals. The particular preparation used was tested separately and 60 to 75 mg. per day provided adequate vitamin B for growth. The use of this concentrate avoided a change in the dietary protein level. The records of these animals (chart 1, tables 2 and 3) contain only two indications that they derived any benefit from the concentrate. The males grew at a more rapid rate and the females succeeded in weaning about 4 per cent of their young. On the other hand, the females made only slightly better gains, had irregular oestrous cycles, and the few young which they reared were subnormal in weight at 21 days of age. Both sexes utilized their food uneconomically.

A watery liquid derived from the dehydration and extraction process (Bloor, '26, p. 37), in the preparation of the extracted liver, was tested for the factor here concerned. It was fed in $\frac{1}{2}$ gm. equivalents as a supplement to ration A. The records of the animals receiving this preparation, also given in chart 1 and tables 2 and 3, show that except for a

slight improvement in the growth rate of the females the animals did as poorly as the controls receiving diet A alone. Apparently the growth-promoting and lactation factor was destroyed in the process of preparing the extract, or partially destroyed and too small an amount was fed. Mapson's ('32) experiments indicate that the growth factor is water soluble, and our preliminary experiments with other preparations also indicate this.

The results obtained with vitamin concentrates are often confusing and difficult to interpret by various workers. For that reason and also because the several members of the vitamin B complex recently described appear to be present in yeast, studies were made of the effect of varying the amount of yeast. Chart 2, figure 1, shows the effect of supplements of $\frac{1}{2}$ gm. and of 1 gm. of yeast to diet A. The resulting improvement can be ascribed, at least in part, to the yeast protein, for it was shown in the previous paper that a 15 per cent protein level did not give normal growth.

Figure 2 shows the result of a second experiment in which diet A and also C (15 per cent dried whole liver protein) were supplemented with 1 gm. of yeast daily. A marked difference was immediately noticeable; the whole liver produced its usual effects. The difference is particularly striking when one considers that the animals receiving diet C were at a disadvantage, because the value of the yeast protein was opposed by a diminishing increment in growth rate. Nevertheless the animals on the extracted liver made inferior gains. Beginning with the sixtieth day three animals received diet C in place of diet A. They responded to the change immediately and after 40 days, when they were discontinued, their weights were rapidly approaching those of the animals which had received ration C from the start. Their rate of growth was accelerated beyond that usually obtained on ration C at that age. This may be regarded as a resumption of growth after stunting and is a confirmation of the early work of Osborne and Mendel ('15).

In figure 3 of chart 2 are data which confirm and demonstrate more convincingly that yeast does not supply the factor which liver contains. Ten per cent of yeast was included in the basal rations A and C in place of an equal amount of

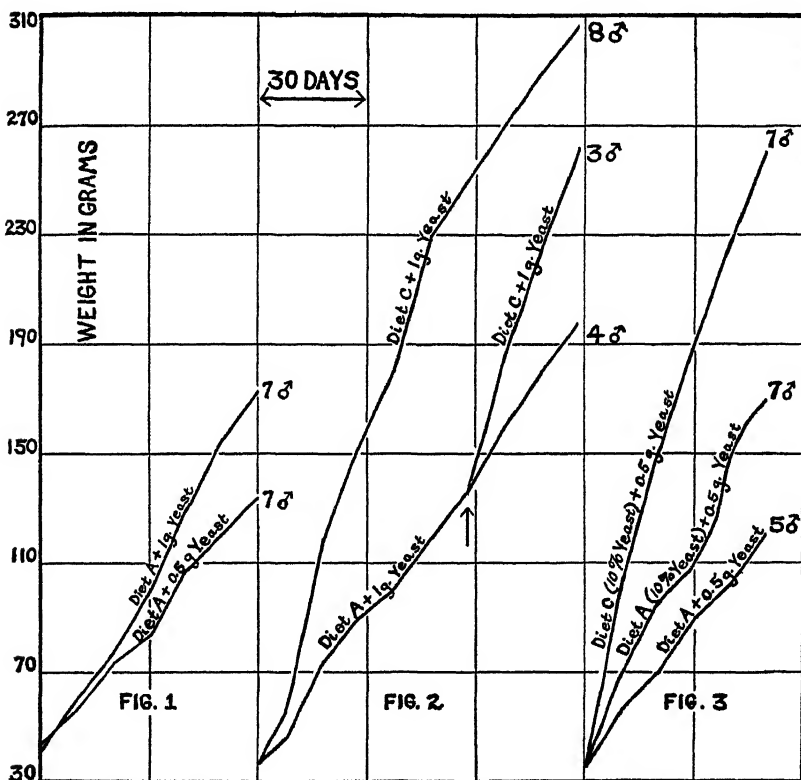


Chart 2 The chart shows the combined growth curves of male albino rats, and illustrates the effect of varying the amount of yeast fed with the extracted-liver ration A, and the dried whole liver ration C. Each animal received 0.5 cc. of cod-liver oil per day.

starch. In order to induce the animals to eat their daily allotment of cod-liver oil an additional $\frac{1}{2}$ gm. of yeast was supplied as a daily supplement. The yeast employed was the dry product of the Northwestern Yeast Company, and portions from a number of shipments were used in these experi-

ments. If the liver factor is present in yeast it must be there in very small amounts, which could be revealed only by feeding yeast on a much higher level.

In the earlier work a special effort was made to use only freshly dried liver. As the work progressed it soon became apparent that this was not necessary. The dried whole liver used in the experiments recorded in chart 2 had been stored at ice-box temperature for a year and a half. The practical significance of this is obvious. Likewise, no evidence has been obtained which indicates a seasonal variation in the nutritive value of the tissue.

DISCUSSION AND SUMMARY

These observations confirm the conclusions arrived at in the previous paper and leave no doubt that alcohol extraction removes material from liver which is necessary for optimum growth and lactation. They further show that this material is not associated with the liver lipids and that the additional protein consumed when whole liver supplements are fed is not the effective agent. The principle which accelerates growth may be identical with the lactation factor; more than 0.5 gm. raw liver or its equivalent of dried whole liver is necessary for maximum response in each case, and heat treatment has a parallel effect. The superiority of raw liver over dried whole liver for lactation was also observed by Clayton ('30), and appears to be a quantitative rather than qualitative difference.

The multiple nature of the vitamin B complex leads to the easy assumption that the valuable contribution made by whole liver may be one of the several constituents of this complex. Though partially indirect, the evidence presented seems to differentiate this thermostable dietary factor from those present in yeast as well as from any of the hitherto known dietary essentials, and substantiates the conclusions reached by Smith ('31) in a preliminary report of this work.

Mapson ('32) has recently drawn similar conclusions from results obtained by quite different experimental procedures

and has tentatively proposed the name 'physin' for this factor. Physiologically improper proportions of the accepted dietary requisites are known to produce nutritional abnormalities and this new factor may yet prove to be a hypothetical substance, but the failure to obtain optimum growth and lactation on extracted-liver rations, when the known requirements are supplied in abundance, presents a contrast to the relatively small amount of whole liver which will restore these functions to normal.

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IRON METABOLISM STUDIES IN A NORMAL SUBJECT AND IN A POLYCYTHEMIC PATIENT

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TWO FIGURES

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This report presents the results obtained in a study over a period of 7 months of the total iron intake and output of two subjects, one normal and one suffering from polycythemia vera. During this investigation red blood cell counts and hemoglobin determinations were made twice a week.

The patients were confined to the Metabolic Ward of the Russell Sage Institute of Pathology where food was prepared under special precautions. Distilled water was used for cooking, drinking and final rinsing of utensils. All cutlery used was plated and all cooking utensils were aluminum or agate. Care was taken to avoid chipping of the agate. Aliquot portions of all foods were analyzed as described in a publication (Toscani and Reznikoff, '34) appearing in this volume, so that even canned foods were checked against possible iron contamination. Iron was determined by the Elvehjem ('30) and Kennedy ('27) methods and blood counts were done by the same individual with standardized pipettes. Fifteen and eight-tenths grams of hemoglobin per 100 cc. of blood was considered 100 per cent.

¹ The authors desire to express their thanks to the Misses Mabel Quinto, Janet Costello and Thelma Springer for their cooperation.

The urine and stools of the subjects were collected quantitatively and 4-day periods of the excreta were examined at a time. On a few rare occasions 3-day periods were used. In the case of the normal individual the shortest experimental phase was 2 periods; the longest 7; and the average 4. The shortest experimental phase for the polycythemic patient was 2 periods; the longest 12; and the average 5.

The normal subject was a middle-aged male who had come into the hospital complaining of diarrhoea and showed evidences of slight malnutrition and pseudopellagra. After several weeks in the regular ward he had completely recovered and when he was transferred to the metabolic ward was apparently normal. Table 1 and chart 1 give a composite picture of the results obtained with this normal subject.

A summary of the findings shows that this normal subject had a positive balance of more than 5 mg. of iron daily on an intake of about 26 mg. After parenteral liver therapy the retention was increased to 13 mg. daily. This positive balance persisted so that on a 19-mg. intake he stored 8 mg. daily. When he was placed on a 10-mg. intake he was practically in balance. Now parenteral liver therapy, on the same 10-mg. intake, gave him a retention of almost 3 mg. a day. After the possible effect of the liver wore off he again went on approximately 10 mg. ingestion and was in iron balance. On 17-mg. intake he showed storage of about 2.5 mg. daily. A liver extract, supposedly effective in secondary anemia (Whipple, Robscheit-Robbins and Walden, '30), containing a daily iron ration of 60 mg. and, when given with 17 mg. in food, seemed to give a retention of 17 mg. daily. A control period of 17.5 mg. alone gave a balanced condition. Iron alone, in the same quantities as the liver extract contained, added to that in the diet gave a retention of only 6.5 mg. When the patient was now placed on 80-mg. intake and parenteral liver extract was given he showed a rather surprising loss of about 30 mg. of iron daily. At this point it might be of significance that from the start of the experiment until the beginning of this negative phase the subject stored a total of 1388.6 mg. of iron.

TABLE 1

NUMBER OF PERIODS	AVERAGE DAILY IRON INTAKE (MG.)		AVERAGE DAILY TOTAL IRON EXCRETION (MG.)	AVERAGE DAILY IRON BALANCE (MG.)	BLOOD COUNT		COMMENTS
	Food	Medication			Hb. per cent (15.8 gm. = 100 per cent)	R.B.C. in millions	
5	19.4	7.0 (yeast)	20.7	+ 5.7	76 78	4.9 5.1	Lederle's parenteral liver extract, 9 cc. in first period
9	19.4	7.0 (yeast)	13.3	+13.1	75 73 86	4.9 4.4 5.0	
2	19.2		11.1	+ 8.1	81 84	5.0 4.7	
3	10.0		9.3	+ 0.7	92 104	4.8 4.8	
7	10.1		7.3	+ 2.8	100 92 107	4.6 5.1 4.4	
3	10.8		10.9	— 0.1	96 83	4.9 4.2	Lederle's parenteral liver extract, 6 cc. in first period
2	16.8		14.3	+ 2.5	90 99	4.8 4.6	
6	16.8	60.0 (liver extract)	59.8	+17.0	88 90	4.5 5.0	
2	17.4		17.9	— 0.5	87 75	4.3 4.4	
4	17.2	60.0 (iron & ammonium citrate)	70.7	+ 6.5	82 86	4.4 4.9	
5	17.9	60.0 (iron & ammonium citrate)	107.9	—30.0	90 81	4.5 4.0	Lederle's parenteral liver extract, 9 cc. in first period
4	17.8	60.0 (iron & ammonium citrate)	86.1	— 8.3	69 75	4.4 4.9	
3	19.1	2000.0 (iron & ammonium citrate)	1952.5	+66.6	81 96	4.2 5.0	3 gm. of NH_4Cl daily in last two periods

In connection with this apparent reversal of effect of liver extract it is interesting to note that Beckmann ('30) found that in secondary anemia liver caused a marked retention of iron. Riecker ('30) has pointed out that in a remission of pernicious anemia there is an increase in excretion over intake of iron. Therefore the effect of liver extract on iron balance depends to a considerable extent on the relation between tis-

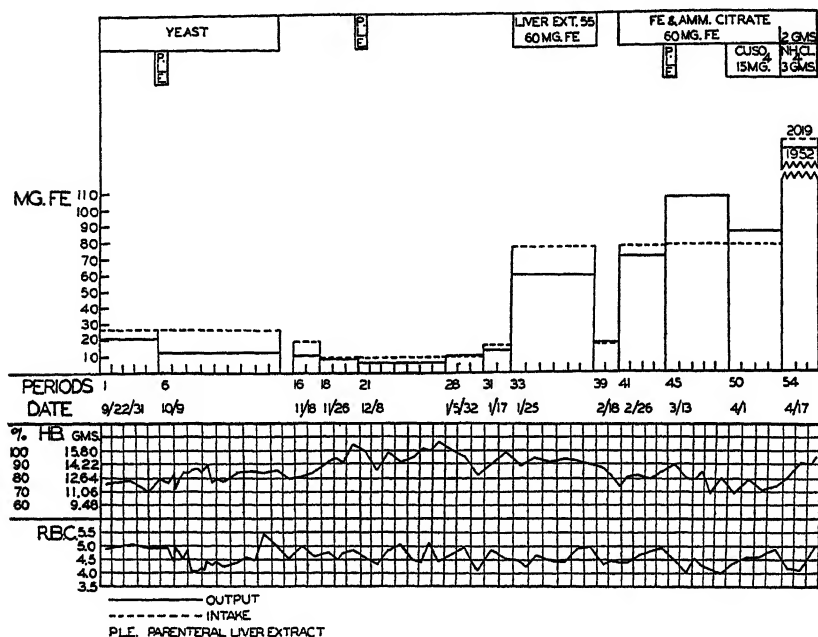


Chart 1. Normal subject

sue iron, hemoglobin iron and ingestion and excretion at any particular time, probably as Josephs ('32) indicates. Copper sulfate apparently cut down this iron loss to 8.3 mg. daily. Josephs ('32) found that copper has no effect on iron retention and tends to shift iron above a basal concentration from the tissues to hemoglobin formation. It is true in this experiment that during the copper administration there was some increase of red cell count and hemoglobin. It is interesting to note that massive doses of iron and NH_4Cl (Aub et al., '26)

had no effect on the urinary excretion of iron in view of Riecker's report ('30) of high concentrations of iron in the urine.

Red cell and hemoglobin studies in this normal individual seemed to show at first a striking independence of variations in iron intake. This is not surprising when one realizes that the storehouses for iron, even in anemia, have an ability to retain iron despite the apparent demands of the blood. However, it can be seen that adequate iron intake, 20 mg. daily, maintained a count of about 5,000,000 and a hemoglobin value of 85 to 100 per cent. Immediately after parenteral liver extract was given a short period of diminished hemoglobin and erythrocyte count was obtained. This is not infrequently seen in liver therapy in pernicious anemia. When the subject was placed on a 10-mg. intake his red cell count decreased to 4,200,000 and his hemoglobin to 83 per cent. This rapidly increased on a higher iron intake. After his period of marked negative balance his count again decreased to 4,000,000 and between 70 and 80 per cent hemoglobin. But on discharge after a CuSO_4 and a very high iron intake phase his final count was 5,000,000 R.B.C., 96 per cent hemoglobin. It is of interest to note that his initial hemoglobin value was 76 per cent of 15.8 gm. His total retention of iron throughout the experiment was 1413.2 mg. If converted entirely into hemoglobin he should have shown an increase in hemoglobin of 6 gm. or 38 per cent per 100 cc. of blood (Bassett, Killip and McCann, '31). This would have given a final hemoglobin reading of 114 per cent if none of this iron were stored but converted entirely into hemoglobin.

The polycythemic patient came into the ward during a remission stage. He had shown 10,000,000 red blood cells a few months previously and had been treated by radiation and phenylhydrazine with good response. When he entered the metabolism ward his red cell count was 5,300,000 and his hemoglobin was 80 per cent. Not until he had been studied for almost 4 months did his red cells begin to increase appreciably so that the effects of therapy on his iron metabolism

could be followed. Therapy consisted first of Fowler's solution according to the method of Forkner ('32). It was found that although his count could be brought down to 5,000,000 the effective dose was so close to the toxic one that medication had to be suspended. At the end of nine periods his red cell count was again 7,000,000 and his hemoglobin rose to 125 per cent.

The patient was now given intensive x-ray therapy, eleven treatments within 24 days. His red cell count dropped to 5,300,000; his hemoglobin to 87 per cent. But 1 week later his count rose to 6,100,000 and 113 per cent hemoglobin.

For two periods the patient was given no medication or treatment. He was now put on large doses of phenylhydrazine, 0.3 gm. every day for 7 days, a total of 2.1 gm. His red cell count dropped in about 2 weeks after cessation of the therapy to 2,900,000 and his hemoglobin to 56 per cent. When he was discharged 24 days after the medication was withdrawn his red cell count was 3,600,000 and his hemoglobin was 59 per cent.

During a preliminary study the subject excreted in his stool during one period as much as 167 mg. of iron daily. This was never found again during the observation and is mentioned here because it is possible that stored iron may be excreted in large amounts over short periods of time in polycythemic patients.

Table 2 and chart 2 summarize the findings obtained in this patient.

One of the striking features of the polycythemic patient's findings was a lack of correlation between iron intake and iron storage. On a higher intake he stored less iron than on a relatively lower intake at the start of the experiment. Of course we have no knowledge of the state of his storehouses before the experiment began except that in the preliminary tests he excreted relatively large amounts of iron. During his periods of therapy he was constantly on a slightly negative balance but even with a tremendous decrease of 50 per cent of his red blood cells and hemoglobin which occurred with

phenylhydrazine administration his output of iron was insignificant. This substantiates fully the findings of Bassett, Killip and McCann ('31). He actually lost during this period 79.2 mg. of iron. To account for his blood drop he should

TABLE 2

NUMBER OF PERIODS	AVERAGE DAILY IRON INTAKE (MG.)	AVERAGE DAILY TOTAL EXCRETION (MG.)	AVERAGE DAILY IRON BALANCE (MG.)	BLOOD COUNT		COMMENTS
				Hb. per cent (15.8 gm. = 100 per cent)	R.B.C. in millions	
5	12.8	9.6	+3.2	90	5.5	
				83	4.8	
				76	5.5	
3	14.1	15.7	-1.6	84	4.8	
				93	5.1	
6	12.1	8.9	+3.2	99	5.3	
				95	5.8	
12	18.1	16.7	+1.4	100	5.6	
				105	6.1	
				105	7.4	
				110	6.8	
10	17.6	17.9	-0.3	99	6.5	Fowler's solution daily for seven periods; very little in last two periods
				120	5.0	
				118	7.0	
6	18.6	22.1	-3.5	95	6.5	X-ray, 11 treatments in 24 days
				95	5.5	
2	19.1	20.5	-1.4	87	5.3	Phenylhydrazine, 0.3 gm. daily for 7 days in first two periods
				113	5.5	
8	15.9	19.2	-3.3	88	6.1	
				56	2.9	
				59	3.6	

have lost 2100 mg. of iron. This difference was undoubtedly stored.

One of the features of this experiment is the constancy of iron excretion per gram of dried stool. In the normal subject on an iron intake between 10 and 25 mg. the dried stool per

gram varied in iron content between 0.36 mg. and 0.82 mg. with an average of 0.6 mg. On an 80-mg. intake the variation was between 38.4 and 64.0, averaging 48.7 mg. In the case of the polycythemic patient all through the experiment except in the preliminary phase, the variation of iron per gram of dried stool was between 0.32 mg. and 0.78 mg., with an average of 0.51 mg.

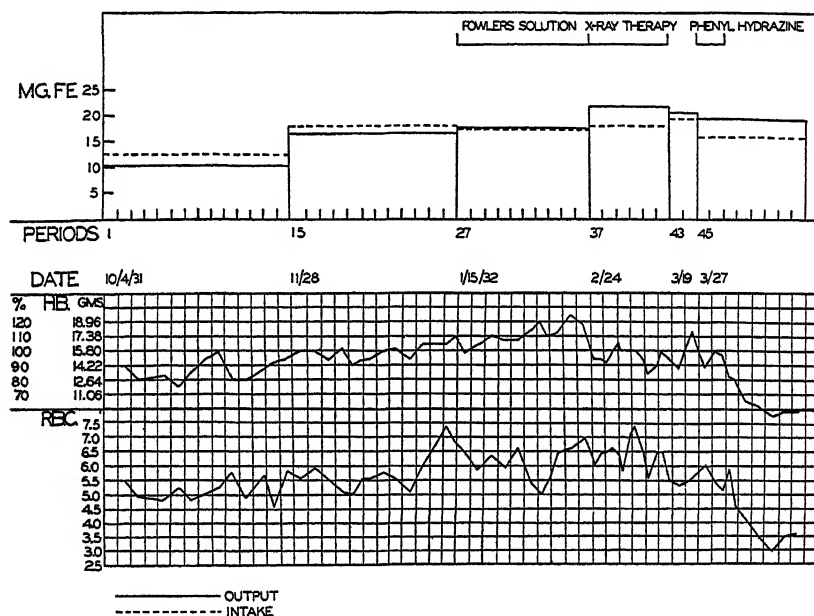


Chart 2. Polycythemic patient

The daily urine excretion of iron in the normal person varied from 0.2 mg. to 0.62 mg., averaging 0.35 mg. In the polycythemic patient daily iron urinary excretion varied from 0.24 mg. to 0.74 mg., averaging 0.42 mg.

CONCLUSIONS

1. A normal subject was found to store iron when placed on an intake of 26 mg. daily and was in balance when on a 10-mg. intake before any therapy was started.

2. A parenteral liver extract, which is potent in causing a remission in pernicious anemia, appeared to cause a retention of iron when the subject was on a 10-mg. intake and an increase in iron storage when he was on a 17-mg. intake. When he was put on a high iron intake, approximately 80 mg., administration of parenteral liver extract was followed by a marked increase of iron excretion. Copper sulfate seemed to decrease the iron loss.

3. Administration of a liver fraction, supposedly potent in 'secondary' anemia, was followed by a retention of iron over and above that obtained when iron in quantities present in the liver fraction alone was given.

4. Administration of massive doses of iron (2 gm.) gave a marked retention of iron.

5. During a remission stage a polycythemic patient was on a slightly positive iron balance when his intake varied from 14 to 18 mg.

6. Even with a decrease of 50 per cent of his blood following phenylhydrazine no marked increase of iron excretion was obtained. This suggests that the great bulk of iron so liberated is stored in the body.

7. Iron excretion in the urine is little and remarkably constant and cannot be increased by any method tried in these experiments.

8. The amount of iron excreted per gram of dried stool is remarkably constant except when large amounts of iron are given.

9. The red blood cell count and hemoglobin values had only an indirect relationship to iron intake and therapy in the normal or polycythemic subject because of the intermediation of the iron storage depots.

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HUMAN MILK STUDIES

XII. THE VITAMIN B AND VITAMIN G CONTENT BEFORE AND DURING MATERNAL CONSUMPTION OF YEAST ¹

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TWO FIGURES

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The vitamin B potency of either human or cow's milk alone may not always be sufficient for the consistent growth and well-being of the infant (Hoobler, '28; Rose, '30). Shortcomings are recognized by the appearance of symptoms in the child, varying from the loss of appetite and failure to gain in weight to the manifestation of a definite syndrome of beriberi, symbolizing the lack of the vitamin B complex. Moreover, clinical observations have shown that alleviation of these symptoms follows either the administration of this nutrient, extract of vegetable soup (Daniels, Byfield and Laughlin, '19), wheat germ sugar (Dennett, '29; Waring, '29, '31; Henricka, '32; West, '29; and Berney, '31), or, yeast (Waring, '29, '31; West, '29; Bloxsom, '29) to the infant directly or indirectly by fortification of the diet of the mother (Henricka, '32; West, '29; Brenneman, '23). Since this vitamin moiety can neither be synthesized nor stored in the

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² The data comprise the dissertation submitted in partial fulfillment of the requirements for the degree of doctor of philosophy in the Division of the Biological Sciences, The University of Chicago.

body to any appreciable extent, the maternal diet then must serve not only as the sole supply for maintenance and increased physiological demands of lactation, but also for the vitamin content of the milk.

Although the optimal vitamin requirement of the infant is not known in specific quantitative units, the significant fact is that dietary insufficiencies actually occur in some infants fed either with breast or cow's milk. In many of the clinical studies on human milk the diets of the mothers were known to be notably inadequate. But even the milk from women consuming diets with average amounts of the vitamin B complex has been found by biological assay to contain a relatively low concentration of this substance (Macy et al., '27). Improvement may be effected also in cow's milk formulae fed routinely in the hospital by the addition of specific vitamin adjuvants, as evidenced by the stimulation of a better appetite and increased growth in the infant. These demonstrations that the infant may experience frank dietary deficiencies with milk as the chief source of food, and that there are, no doubt, borderline conditions with symptoms yet unclassified or even unrecognized make it evident that positive means should be devised for insuring the child against alimental inadequacies during the critical period of rapid growth, thereby safeguarding not only present, but future nutritional stability.

It has been demonstrated experimentally that the maternal requirement for vitamin B is augmented during lactation (Macy et al., '27; Evans and Burr, '28; Sure, '27) from three to five times over and above that during growth. In the human mother with a failing or small milk supply, the addition of yeast and yeast preparations to the diet has been observed to promote an increased flow of milk (Waring, '31; Henricka, '32; and Wachtel, '30). On the other hand, women who were successful in the production of large quantities of breast milk experienced no greater output with supplementation of their diet, but a quantitative assay of the biological potency of the milk disclosed an improvement in its vitamin content (McCosh, Macy, and Hunscher, '31). At the same

time the mothers themselves expressed a feeling of lessened fatigue with a general stimulation of well-being (Macy et al., '30).

The augmentation of the diet of the mother with yeast or yeast preparations benefits both mother and child, and since it is recognized that there are at least two well-defined water-soluble vitamins in yeast, it is pertinent to question whether each bears equal influence in the improved condition or whether one portion is more helpful than the other. Laboratory experimentation has indicated that for successful lactation and rearing of young rats, increased amounts of both the antineuritic vitamin B and of vitamin G are needed (Sure, '28). Some investigators have found a greater need for the former (Evans and Burr, '28) and others for the latter (Husseman, '31), but no study as yet has been reported on women. There are clinical observations, however, indicating that the rapidly growing infant requires larger amounts of each of these vitamins than is furnished by milk, for the feeding of accessory food in addition to the milk resulted in some cases in a promotion of growth in the infant with no increase in total food consumption (Hess, '17), in others the appetite was stimulated as well as growth, while in others the symptoms of rigidity and beriberi were mitigated. The improvement in the infant accompanying the enrichment of the maternal dietary may be due to a selective augmentation of one or the other or both of these two well-recognized vitamin constituents or to some unidentified factor in breast milk. From experimental observations in this laboratory (McCosh, Macy, and Hunscher, '31) there are indications that the fractions may be increased disproportionately. When the average diets of lactating women were supplemented with yeast, some factor promoting better utilization of food was increased in the milk. The factual evidence was demonstrated by the behavior of experimental animals in that equal or better gains in body weight were achieved on fewer calories per square meter of surface area for 24 hours during the period of maternal yeast consumption.

Since the vitamin requirement for lactation has not been measured quantitatively for woman, and there is experimental evidence to indicate that the demand for the vitamin B complex is increased at this time, further enrichment of the average dietary in this essential food component may approach a more nearly optimal food provision for both the mother and her dependent infant. By a quantitative assay of each of the constituents of this vitamin moiety passed into milk, concomitant with the supplemented maternal dietary, the ingredient which is instrumental in improving the nutritive potency of the milk may be recognized. Data derived from such an assay may aid in assuring "a more nearly perfect food for the infant," one which will supply all of the nutritive needs of the young child, thus stimulating his optimal physical progress.

The present report is a study of the effectiveness of diets rich in the vitamin B complex on the nutrition of women during lactation and on the biological potency of the milk secreted coincidentally. It includes quantitative tests on the vitamin B and vitamin G³ content of breast milk of an individual as well as of mixed milk from a group of women before and after their accustomed menus were supplemented with yeast. In addition, data are given on the relation of growth of experimental animals to the vitamin and total caloric ingesta, as well as on the composition of growth as measured by nitrogen balance studies.

EXPERIMENTAL

Subjects and breast milk. The women chosen to provide the milk for this investigation were able to secrete enough to feed their own infants and to supply for the Mother's Milk Bureau of Detroit approximately 13 ounces in excess daily. Observa-

³ The terms for the different vitamins as used in this report are defined below for clarity in discussion; vitamin B complex is the undifferentiated vitamin originally designated as vitamin B; vitamin B is the antineuritic substance for which the British use the term vitamin B₁; vitamin G is the more heat-stable substance or substances known originally as the 'Goldberger Factor' and which is specified as vitamin B₂ by the British.

tions were begun in the second or third month of lactation and extended over a period of 6 to 8 months thereafter, during which time they maintained their usual output of milk. The maternal food and liquid intakes were measured 1 week out of each month. All of the diets during the period of observation were found to be adequate according to present accepted qualitative dietary standards (Sherman, '32). The milk secreted during the period in which the women were eating their accustomed diets and that produced after 6 weeks of consumption of supplements of 10 gm. of yeast daily were tested for vitamin B and vitamin G potency.⁴ At 9 o'clock each morning the milk was manually expressed at the Bureau, and the containers sealed air tight and packed in ice until the milk was fed at 11 A.M. The yeast supplement was administered at the Mother's Milk Bureau. The infants of the women were healthy throughout the observation and made for the most part average gains of about 6 ounces per week.

For statistical interpretation and the assurance of representative growth, large numbers of test animals are required for biological assay work, consequently a greater amount of test material must be available. The quantity factor becomes more significant in testing mother's milk for vitamins B and G, since not only must large numbers of animals be maintained, but considerable amounts of milk are required for the several graded levels fed. Human mothers, as a rule, do not produce much milk in excess of that needed by their own babies, hence, in order to secure a sufficiency for reliable biological tests, pooled milk from a group of women must be used, although certain inherent complications are introduced. In view of the fact that there seems to be a direct relationship between the concentration of the vitamin B complex and the quantity of milk secreted (McCosh, Macy, and Hunscher, '31; Macy, Outhouse, and Hunscher, '28), it is of

⁴For convenience the period during which the mothers were on their usual diets will be designated as the pre-yeast period and that following and during the addition of yeast to the maternal diet as the yeast period. The vitamin G potency of 10 gm. of yeast is equivalent to 75 to 150 units, according to Sherman.

importance in a study of potency of the vitamins B and G in mixed milk, and in particular the effectiveness of dietary supplements upon the biological properties, to secure women of comparable capacities for milk production. In the mixed sample, a change in the vitamin concentration of one individual milk may be concealed by the diluting effect of the lack of change in another. This indicates the advisability of studying individual milks as a control even though it becomes necessary to use fewer samples because of the diminished quantity of available sample. In this study not only mixed milk combined under standard conditions but also the milk of an individual mother as a control was used.

Experimental animal technic. Young albino rats reared on a stock diet of whole wheat and whole milk powder (Sherman and Campbell, '24) were placed in individual cages at approximately 28 days of age and given weighed portions of the experimental basal diet which they were allowed to consume ad libitum, except in one series of studies in which the quantity was controlled. The basal diet (Chase and Sherman, '31) fed for determining the vitamin B content of milk contained casein 18, dextrin 53, autoclaved yeast 15, filtered butterfat 8, cod liver oil 2,⁵ Osborne and Mendel salt mixture 4 (Osborne and Mendel, '19); and for determining vitamin G (Bourquin and Sherman, '31) casein 18, butterfat 8, cod liver oil 2, Osborne and Mendel salt mixture 4, and dextrin 68, on which was dried an alcoholic extract of whole wheat in such proportion as to introduce the extract (80 per cent alcohol by weight) of 50 gm. of whole wheat into 100 gm. of food mixture. For the most part from six to ten standardized animals were maintained on each test level of milk over an 8-week experimental period following a period on the vitamin-free diet during which the exhaustion of the store of vitamins B and G in the animal body took place.

Only those animals that consumed all of the milk daily are included in the final averages of the weekly weight increments. It happens that the milk of some women is eaten with greater

⁵ The kindness of Arthur D. Holmes, Ph.D., of the E. L. Patch Company, Boston, in supplying the cod liver oil is gratefully acknowledged.

avidity than that of others and this distaste of some animals for the milk persists throughout the test period, thus precluding the use of these results in the final interpretation of the data. With the larger quantities of breast milk as the source of the antineuritic vitamin, diarrhea frequently occurs in contrast to almost complete freedom from this condition with analogous quantities of milk supplying vitamin G. This fact is significant and is in accord with the findings that debilitation and lessened muscular tone of the intestinal tract accompany sub-optimal intakes of the antineuritic vitamin

TABLE 1

LEVEL OF MILK	PRE-YEAST PERIOD				YEAST PERIOD			
	Number of animals	Mean weekly gain	Mean weekly basal food intake	Mean weekly gain 100 cal. food	Number of animals	Mean weekly gain	Mean weekly basal food intake	Mean weekly gain 100 cal. food
Vitamin B-free diet + mixed breast milk								
<i>Cc.</i>		<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>		<i>Gm.</i>		
10	10	2.5	28.8	1.8	6	2.7	31.5	1.6
20	10	8.5	31.4	3.4	6	7.4	36.0	3.1
25	5	8.9	28.7	3.4	7	8.7	26.3	3.6
Vitamin G-free diet + mixed breast milk								
3	10	2.0	27.3	1.4	8	2.9	30.5	1.9
5	10	2.5	26.8	1.8	8	6.0	36.0	3.2
10	10	7.8	35.3	3.6	7	7.3	35.6	3.4
20	12	11.5	44.3	3.7	7	9.6	30.3	3.9

(Rose, Stucky and Cowgill, '30; McCarrison, '21). The high concentration of lactose in mother's milk may also be a mediating factor (Macy et al., '27). Because of these vitiating conditions, tests on the antineuritic vitamin potency of human milk are less conclusive than those on the vitamin G content.

Basal diet ad libitum. Equal quantities of breast milk from women with similar production records were combined daily to form the sample of mixed milk. Individual standardized test animals were given varying quantities of milk from 10 to 25 cc. daily as the sole supply of the antineuritic vitamin in a diet otherwise complete, and 3 to 20 cc. for the vitamin G test (table 1). The smaller quantities allow for the deter-

mination of the unit growth suggested by Sherman (Chase and Sherman, '31; Bourquin and Sherman, '31) and the increased amounts permit a study of the influence of more adequate vitamin intakes.

In both the vitamin B and vitamin G studies there was a progressive increase in the growth of the test animals coincident with increased consumption of milk as a source of these vitamins. This was true whether the milk was secreted by the mothers in the pre-yeast period or in the yeast period when supplements of 10 gm. of yeast were being consumed daily along with their ordinary diets.

With 3, 5 and 10 cc. of mixed milk daily as the source of vitamin G, the growth of the animals in the yeast period was equal to, or superior to, that of the pre-yeast period. The mean weekly gain of the standardized test animals over an 8-week period for the daily intakes of 3, 5, 10, and 20 cc. of mixed breast milk was 2.0, 2.5, 7.8, and 11.5 gm., respectively, for the animals in the pre-yeast period and 2.9, 6.0, 7.3, and 9.6 gm., respectively, for the yeast period. The increased gain of the animals when they were receiving milk at the lower levels from the mothers consuming a dietary supplement of yeast demonstrates that the vitamin G is carried over into the milk in appreciable quantities. These findings are further borne out by a study of the milk from an individual woman in which case the mean weekly gain in weight of animals receiving 3, 5, and 10 cc. of milk daily was 0.3, 3.4, and 7.0 gm. for the pre-yeast period and 3.6, 4.2, 7.9 gm., respectively, for the yeast period. Divergent results were at times experienced when quantities of 20 cc. of milk daily were fed for vitamin G. These inconsistencies with the higher level of milk may possibly be ascribed to the exhaustion of the store in the animal body of some yet unidentified food factors necessary for the growth of the rat as suggested by Bourquin and Sherman ('31) and hence these results may not be as reliable as those for the lesser quantities fed.

In contrast to the experiments on the vitamin G content of breast milk, the ingestion of yeast by the mothers seems to

exert no perceptible influence on the concentration of vitamin B in the milk secreted. Animals getting quantities of breast milk as high as 10 cc. daily developed polyneuritis in approximately one-quarter of the cases both before and after supplemental additions of yeast to the maternal dietary. On higher levels of milk, 20 and 25 cc. daily, results were complicated by failure to take all of the milk and also by the occurrence of diarrhea. The mean weekly gains of those animals consuming the full quota of milk daily, irrespective of other conditions, were for the animals in the pre-yeast period getting 10, 20, and 25 cc. of mixed milk, respectively, 2.5, 8.5, and 8.9 gm., and for the comparable animals in the yeast period, 2.7, 7.4, and 8.7 gm. After eliminating from the final average the gains of all animals ill-conditioned either by the failure to consume all of the milk daily or by the diarrheal condition, the resultant mean weekly gains for the remaining animals in the pre-yeast group getting 10, 20, and 25 cc. of milk daily were, respectively, 2.5, 8.8, and 10.4 gm., and for the animals in the yeast period 2.7, 9.0, and 10.6 gm. As judged by the rate of growth of rats, no discernible increase in the antineuritic potency of the breast milk herein studied was effected by the addition of 10 gm. of yeast to the maternal dietary of average vitamin content.

The weekly gain of the test animals was studied in relation to their caloric food consumption in order to further verify the growth rates of the animals in the pre-yeast vs. the yeast period, and to ascertain whether these increments were the result of a difference in the utilization of food or of an increased food intake. As based upon gain per 100 calories of food consumed, the test animals getting vitamin G from mixed milk at the lower levels manifested a noteworthy increase during the yeast period, whereas, no improvement could be recognized in those animals having milk as a source of vitamin B (table 1). These findings are confirmatory of the results of a previous study in this laboratory (McCosh, Macy, and Hunscher, '31) in which a more efficient use of food was similarly observed in experimental animals receiving breast

milk secreted during the addition of yeast to the diet of lactating women. The present study demonstrates that this improvement was due to the augmentation of the vitamin G fraction.

With enrichment of the maternal diet with 10 gm. of yeast daily, there occurred an increase in the vitamin G content of the breast milk, but there was no detectable improvement in the vitamin B potency. The vitamin G content was approximately 0.2 unit per cubic centimeter and the vitamin B concentration 0.1 unit per cubic centimeter before the supplementation of the mother's diet, but after the maternal consumption of yeast the concentration of vitamin G in the milk was increased to 0.3 unit per cubic centimeter. The specific causal factors for the disproportionate improvement in these biological components of the milk is enigmatical, and deserves detailed investigation. From their work on cows and goats, Gunderson and Steenbock ('32) concluded that the maximum concentration of vitamin B in milk is under physiological control, for when the content of this vitamin in the ration was increased from a reasonably good level to approximately 66 per cent more by the addition of yeast and wheat germ, the concentration of the antineuritic factor in the milk was unchanged. A similar interpretation may be applicable to the observed constancy of the vitamin B potency of human milk.

Controlled intake of basal diet. From the growth studies in which the basal food was consumed ad libitum, it was apparent that gain in weight of the experimental animals, although primarily influenced by the quantity of vitamin fed, varied somewhat with the amount of basal food consumed (table 1). Variability in the quantity of basal food intake is inherent in an experiment in which the food is fed ad libitum and which factor Irwin, Nelson et al. ('30) have found to bear a significant influence on total gain in body weight. For this reason a series of observations with two groups of rats of ten each were conducted on the vitamins B and G content of the milk of an individual mother fed at 5 and 15 cc. levels daily before

and during the addition of yeast to her diet in which the total caloric allowance of food for the test animals during the yeast period was maintained at the same level per gram of body weight as during the pre-yeast period. The observation extended over 7 weeks, divided into a 2-week pre-yeast period followed by a 5-week yeast period. During the last 3 weeks of the latter the amount of milk supplied daily to each group of rats was increased to approximate the mean amount fed per gram of body weight during the pre-yeast period, because of the recognition that some relationship exists between body weight and the vitamin B requirement (Osborne and Mendel, '22; Cowgill et al., '32). Under these controlled conditions there was a marked acceleration in the growth of animals having vitamin G supplied by breast milk during the ingestion of yeast by the mother; whereas, the rate of gain in body weight of the animals with milk as a source of vitamin B was not significantly changed (chart 1). The yeast supplementation had been made to the mother's diet only 2 weeks prior to the experimental observations—a shorter length of time than in the other studies of this report—but even in this case there was an increase in the vitamin G potency of the milk as judged from the marked increase in gain in body weight of the test animals.

Human milk, as determined by quantitative biological tests, is a comparatively richer source of vitamin G than of vitamin B. With the same quantity of breast milk supplying each of these factors in the respective vitamin-free diets, the growth of the animals with vitamin G supplied by milk was superior to that of those with milk as the source of vitamin B (chart 2). By relating the increment in weight of each group of test rats to their food intake (gain per 100 calories of food consumed) the growth of those animals having vitamin G from breast milk surpassed that of those receiving the antineuritic vitamin from the same amount of milk irrespective of the maternal dietary (table 1). The mean weekly gain in body weight per 100 calories of food intake of the animals with 10 cc. of breast milk daily as a source of vitamin B was 1.8 gm. in contrast

to a gain of 3.6 gm. in those rats with milk as a source of vitamin G; the gains during the yeast period were 1.6 and 3.4 gm., respectively. With 20 cc. of breast milk daily supply-

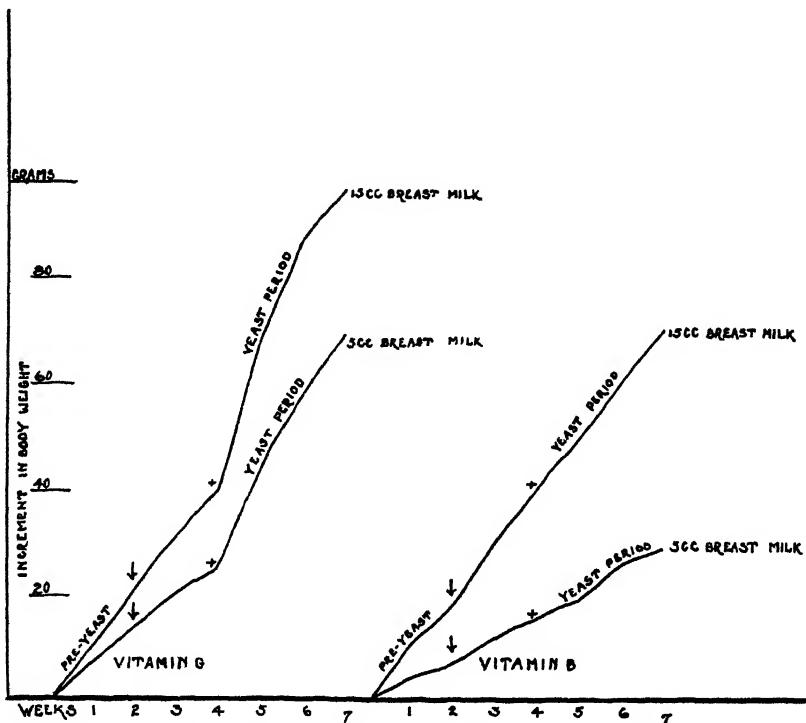


Chart 1 This chart illustrates the average gain in weight of groups of test rats maintained on an approximately constant caloric food intake per gram of body weight with 5 and 15 cc. of milk daily per rat from an individual woman as the source of vitamins B and G in a diet otherwise complete during the pre-yeast period and the equivalent amounts in terms of grams of body weight to compensate for the increased size of the animal during the yeast period. The arrow indicates the introduction of 10 gm. of yeast daily into the maternal diet.

ing vitamins B and G, the mean weekly gains of experimental animals per 100 calories of food consumed were 3.4 and 3.7 gm., respectively, during the pre-yeast period and 3.1 and 3.9 gm., respectively, during the yeast period.

A quantitative comparison of the vitamin B and G content of breast milk is possible by ascertaining the amounts of milk required as a source of each of these vitamins to support unit growth (an average gain of 3 gm. per week for 8 weeks). As a source of vitamin B, 10 cc. of milk gives an average gain in weight per the unit rate in the experimental animals during

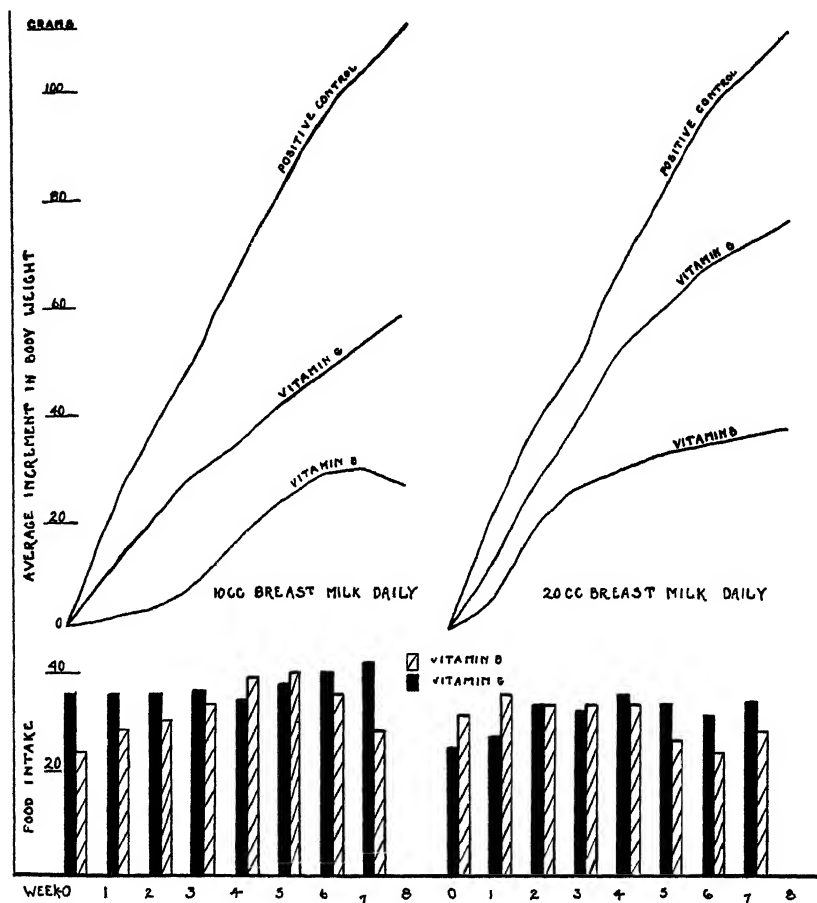


Chart 2 This chart compares the vitamin B and G potency of breast milk. In neither case, however, did the animals receiving as much as 20 cc. of milk daily per rat grow as satisfactorily as the positive controls. The animals consuming milk as the source of vitamin B ate somewhat less basal food than those in the vitamin G test.

both the pre-yeast and the yeast period, despite the occurrence of polyneuritis in approximately one-quarter of the cases, whereas, only 5 cc. daily are required for unit growth in the vitamin G test animals before supplementation of the maternal diet, and only 3 cc. after the introduction of yeast into the diets of the mothers.

Composition of growth of animals. Since the composition of growth is known to be variable, it seemed pertinent to investigate the type of gain in weight made by test animals receiving vitamins B and G from breast milk secreted before and during enrichment of the maternal diet in these factors. By means of 7-day nitrogen metabolic balance observations,

TABLE 2
Retention of nitrogen in per cent of intake

WEEK	VITAMIN B-FREE DIET + MILK				VITAMIN G-FREE DIET + MILK					
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
1	21	39	37	43	42	65	29	61	42	57
2	31	40	24	44	54	57	30	58	54	50
			No							
3	Loss	46	balance	47	43	49	35	55	43	54
4	Loss	38	9	33	38	42	33	48	38	44
5	14	46	11	34	37	41	35	53	37	43
Mean	22	42	20	40	43	51	32	55	43	50

the milk of an individual woman was studied over 5 continuous weeks while the mother was on her usual dietary, and followed by a study of a similar period when she was consuming 10 gm. of yeast daily. Each animal of the latter period was matched as to weight with an animal of the pre-yeast period and was fed both basal food and vitamin in the same proportion per gram of body weight. Two pairs of animals were observed for the vitamin B test and three pairs for the vitamin G comparison. In all cases a greater per cent of the nitrogen intake was retained by the animals receiving milk from the mother during her period of yeast consumption (table 2). Conservation of the ingested nitrogen was accomplished by a lessened urinary nitrogen excretion (table 3) with no consistent

changes occurring in the fecal nitrogen as indicated by the coefficient of digestibility. The mean nitrogen retentions for each gram of gain in body weight for the paired animals in the pre-yeast period and those in the yeast period getting vitamin B from milk were 20.0 and 28.0 mg., 30.0 and 35.0 mg., respectively, and for those fed milk as a source of vitamin G, 34.0 and 38.0 mg., 31.0 and 39.0 mg., and 34.0 and 39.0 mg., respectively. The composition of the growth of the test animals receiving breast milk as the source of vitamin G was of higher nitrogen content during both the pre-yeast and the

TABLE 8
Excretion of nitrogen in urine in per cent of intake

WEEK	VITAMIN B-FREE DIET + MILK				VITAMIN G-FREE DIET + MILK					
	Control	Experi- mental	Control	Experi- mental	Control	Experi- mental	Control	Experi- mental	Control	Experi- mental
1	64	48	46	39	49	25	60	30	49	31
2	52	51	58	35	35	34	56	29	35	38
3	78	45	63	34	47	39	51	34	47	36
4	82	48	70	45	49	44	53	40	49	44
5	60	55	66	49	50	45	51	36	50	46
Mean	67	49	61	40	46	37	54	34	46	39
Coefficient of digestibility	77	87	81	80	89	88	87	89	89	89

yeast period than that of the animals consuming a like quantity of milk for vitamin B.

Since it has been demonstrated that enrichment of the maternal dietary confers added nutritive properties to the milk, it is of interest to know the simultaneous effect that occurs in the metabolism of the mother. Nitrogen balances of lactating women determined in this laboratory before and during the supplementation of their usual diets with yeast indicated that enhancement in the biological properties of the milk did take place (McCosh, Macy, and Hunscher, '31) and in some cases was accompanied by improved storage of nitrogen by the mother herself (Hunscher et al., '33).

SUMMARY

1. Individual and mixed samples of breast milk from women with approximately the same milk flow and on apparently satisfactory dietaries were tested biologically by means of several different controlled methods for their vitamin B and vitamin G potency before and during the supplementary additions of 10 gm. of yeast daily to the accustomed maternal dietary.

2. In both the vitamin B and vitamin G studies there was a progressive increase in the growth of the test animals simultaneously with increased consumption of breast milk. This was true whether the milk was secreted during the pre-yeast period or the yeast period.

3. The concentration of vitamin G in breast milk was increased from 0.2 unit per cubic centimeter during the pre-yeast period to 0.3 unit during the yeast period; an increase of approximately 0.1 unit per cubic centimeter, by the addition of 10 gm. of yeast to the maternal diet for the milk tested.

4. As judged by the rat growth rate method, no increase in the antineuritic potency of breast milk occurred under yeast therapy. The concentration of approximately 0.1 unit of vitamin B per cubic centimeter of breast milk remained unchanged.

5. By a series of observations in which the caloric food consumption of the experimental animals was controlled, further evidence was given that the vitamin G concentration is increased, but that no change occurs in the vitamin B content of breast milk with this enrichment of the maternal diet.

6. Nitrogen metabolic balances on test rats receiving vitamins B and G from breast milk, before and after the addition of yeast to the maternal diet, showed a greater retention of nitrogen during the latter period in all animals. The composition of the growth of the animals with vitamin G from breast milk was higher in nitrogen content than the growth of the animals with vitamin B supplied by equal quantities of milk.

7. From the observations on the women of this investigation, it can be concluded that the biological potency of breast milk can be enhanced by the addition of a concentrated source of the vitamin B complex to the average dietary. At the same time, the mothers themselves experienced less fatigue and a more satisfactory feeling of well being. These studies further suggest the need for more adequate knowledge of diet for lactating women in order that an approach can be made toward a nearly optimal food provision for both the mother and her dependent infant.

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THE INFLUENCE OF PREVIOUS EXERCISE UPON THE METABOLISM, THE RECTAL TEMPERA- TURE, AND THE BODY COMPOSITION OF THE RAT ¹

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Exercise not only causes a marked increase in total metabolism, both during the period of activity and after exercise has ceased, but prolonged, regular exercise alters greatly the chemical composition of the body in that presumably less fat is deposited and hypertrophy of the muscles takes place. These characteristics are accentuated in the trained athlete. Among animals the effects of exercise or lack of exercise are strikingly illustrated by the lean, coursing whippet and the fat, inactive pig. With the rat Asher and Curtis ('25), also v. Árvay and Verzář ('31) have studied the respiratory exchange during a moderate amount of exercise. Our studies dealt with the immediate after-effect of exercise upon the oxygen consumption and the rectal temperature of the rat and also with the effect of prolonged exercise (moderate and severe) upon the metabolism and the body composition.

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METHODS

Male albino rats from the Osborne and Mendel colony were used. Some of them were made to run in motor-driven, revolving cages (Reed et al., '30) of sheet metal. In the experiments on the immediate after-effect of exercise the rats (nos. 3, 5, 7, and 9) were exercised 5486 meters daily for about 85 days prior to the special metabolism measurements. In the study of the effect of prolonged muscular activity, growing rats (nos. 1 to 9 and nos. 19 to 27) were exercised daily beginning at the age of 30 days (60 gm.) and continuing until the rats were 130 to 150 days old. The rats exercised between 8.15 A.M. and 12.15 noon and between 2.30 and 5 P.M. In one series the cages revolved twenty-six times, in another eleven times per minute. One revolution equaled 92 cm. An intermittent timer permitted alternately 2 minutes of running and 1 minute of resting. When not running, the exercised rats lived separately in cylindrical metal cages. The control rats were housed separately. The environmental temperature remained at about 25°C. The exercised rats had access to food and water only when not running. Food was not available to the controls when the exercised animals were running.

Experimental feeding began when the rats weighed 60 gm. (30 days old). The following synthetic diet was fed:

<i>Components</i>	<i>Per cent by weight</i>
Casein,	18
Salt mixture, Osborne and Mendel ('19),	4
Cornstarch,	54
Butter fat,	9
Lard,	15
Calorie value per gram, ²	5.2

This basal diet was supplemented daily with dry brewers' yeast. Rats 1 to 18 also received cod liver oil. The technic of feeding and estimating food consumption was essentially that described by Ferry ('20).

The metabolism was measured with a multiple-chamber respiration apparatus (Benedict, '30). When the rats weighed

² The conventional caloric values (protein, 4.1; carbohydrate, 4.1; fat 9.3) were employed.

less than 180 gm. or when short, successive periods were desired (as in measurements immediately after exercise), a small volume of oxygen (about 185 cc.) was introduced into the chamber and the time required for its absorption noted. When the rats weighed 180 gm. or more, the volume introduced was 370 cc. The heat production has been calculated from the measured oxygen consumption (reduced to 0°C., dry, and 760 mm.) on the assumption that the fasting respiratory quotient was 0.72 and that the oxygen consumed had a caloric value of 4.702 calories per liter. The surface area was estimated from the body weight of the rat (in grams) at the close of the metabolism measurement by the formula $S = 9.1 \times w^{2/3}$.

Following the experiments on prolonged exercise, the body composition was determined. Seven rats (approximately 150 days old) and seven controls were killed with illuminating gas after they had fasted (with access to water) 24 hours at 26°C. The fresh weight of the animal, less intestinal contents, was ascertained. The entire animal, including the skin and the intestinal tract (less contents), was put through a food chopper twice. The hashed animal was dried in a partial vacuum at 105°C. After 48 hours of desiccation, the material was ground in a mortar to pulverize pieces of bone. Pieces of skin and hair were cut with scissors. Drying was continued until less than 0.5 gm. of moisture was lost in 12 hours. The moisture content was estimated from the difference in the fresh weight and the weight of the dry tissue. The fat content of the dried tissue was determined by a modification of the Bloor method (Reed et al., '30), the nitrogen by the Kjeldahl method, and the ash by ignition in porcelain crucibles.³ Each result recorded (table 9) represents the average of at least two well-agreeing determinations.

THE IMMEDIATE AFTER-EFFECT OF EXERCISE UPON METABOLISM

The metabolism of four male rats approximately 125 days old was measured immediately after exercise. On the day

³ The authors are indebted to Dr. L. L. Reed, of Yale University, for the fat determinations and to Dr. T. G. Phillips and N. F. Colovos, of the University of New Hampshire, for the ash analyses.

preceding the metabolism measurements these rats were not exercised and, to prevent unnecessary drafts upon the body reserves, they had access to food until 24 hours before the metabolism was measured. For 20 hours before the experiment they lived at an environmental temperature of 27°C. After this preliminary treatment, they each ran 4846 meters in 3½ hours (environmental temperature, 25°C.). In order that the metabolism might be determined as soon as possible after cessation of exercise, the respiration apparatus was maintained in a state of equilibrium prior to the moment of beginning the observation on the exercised rat by keeping a 'dummy' rat (really a normal animal of the same weight as the experimental rat) in the respiration chamber for about 1 hour before the experimental animal was to be measured. Fifteen minutes after the dummy rat had been replaced by the experimental animal, measurement of oxygen consumption (at 30°C.) began. Observations were made usually in ten consecutive periods, varying in length from 20 minutes immediately after exercise to 40 minutes several hours later and extending over 5½ hours. The results are recorded in table 1, expressed as cubic centimeters of oxygen (reduced) consumed per 200 gm. of rat per minute. The time after exercise is calculated to the beginning of the particular period of measurement. The first three consecutive low oxygen values for each rat have been italicized. The activity is recorded as the percentage of time that the rat was active during the period of measurement.

The oxygen consumption was higher 15 minutes after exercise than it was later. One hour after exercise it was still from 8 to 10 per cent higher than in subsequent low periods, and a plateau, not necessarily basal, was not reached until 2 to 4 hours after exercise. The sustained after-effect of exercise upon the metabolism of these rats was not unlike that noted with dogs and man after a comparable amount of exercise.

The metabolism of the individual rats varied from period to period. Only one, no. 9 was uniformly quiet during con-

secutive periods of measurement. The metabolism of this rat was 53 per cent (44 per cent, corrected⁴) higher 10 minutes after exercise than in subsequent low periods in the second

TABLE 1

*The immediate after-effect of exercise upon the metabolism of the rat
(measured at 30°C. and 24 hours after food)*

RAT, AGE, AND WEIGHT	TIME AFTER EXERCISE	LENGTH OF PERIOD	O ₂ PER 200 GM. PER MINUTE ¹	ACTIVITY	RAT, AGE, AND WEIGHT	TIME AFTER EXERCISE	LENGTH OF PERIOD	O ₂ PER 200 GM. PER MINUTE ¹	ACTIVITY
	Hr. Min.	Min.	Cc.	Per cent		Hr. Min.	Min.	Cc.	Per cent
No. 9	10	28	4.37	14	No. 5	15	23	4.65	48
137 days	38	35	3.35	6	137 days	38	26	4.12	27
286 gm.	1 13	37	3.17	5	314 gm.	1 04	32	3.34	13
	1 50	41	<i>2.86</i>	5		1 36	34	3.14	6
	2 31	41	<i>2.85</i>	7		2 10	36	2.96	8
	3 12	41	<i>2.84</i>	12		2 46	31	<i>3.44</i>	35
	3 53	40	2.91	12		3 17	34	<i>3.13</i>	12
	4 33	37	3.14	16		3 51	34	<i>3.12</i>	12
						4 25	37	<i>2.87</i>	3
No. 7	21	20	4.63	25	No. 3	13	28	4.05	14
113 days	41	25	3.80	4	114 days	41	32	3.55	14
354 gm.	1 06	24	3.95	25	296 gm.	1 13	31	3.66	19
	1 30	25	3.79	16		1 44	32	<i>3.54</i>	6
	1 55	23	4.12	35		2 16	34	<i>3.33</i>	18
	2 18	26	3.64	4		2 50	35	<i>3.23</i>	9
	2 44	24	3.94	25		3 25	30	3.75	23
	3 08	25	3.77	16		3 55	32	3.51	0
	3 33	23	4.10	17		4 27	34	3.31	0
	3 56	24	3.92	8		5 01	34	3.22	6
	4 20	26	<i>3.62</i>	15					
	4 46	28	<i>3.36</i>	7					
	5 14	29	<i>3.16</i>	10					

¹ Values in italics represent the first three consecutive periods of low oxygen consumption.

and third hours after exercise, 18 per cent higher 38 minutes after exercise, and 11 per cent higher 1 hour and 13 minutes after exercise. The other three rats were occasionally quiet for short periods, but the general picture is one of excessive

⁴ Preliminary experiments with rats that had not exercised immediately before metabolism was measured, showed that heat production was from 8 to 9 per cent higher in the first 40 minutes after placing the rat in the respiration chamber than in subsequent periods, even if a state of equilibrium had been reached as explained on page 254.

and variable activity during the first 4 hours after exercise. The restlessness, which was reflected in irregular and increased metabolism, was possibly an after-effect of exercise. It cannot be attributed to the time of day when the metabolism was measured, for it occurred between 11 A.M. and 3 P.M. when rats are usually quiet. Neither can it be attributed to the nature of the rat. When the metabolism of the same animals was measured 40 hours after exercise previous and subsequent to the special measurement immediately after exercise, the rats were seldom active more than 15 per cent of the total 4 hours of observation.

TABLE 2

A comparison of the metabolism of rats 2 to 4 hours after exercise with that 40 hours after exercise (measured at 30°C. and 24 hours after food)

RAT	AGE	BODY WEIGHT	DAYS OF EXERCISE	HOURS AFTER EXERCISE	CALORIES PER SQ.M. PER 24 HOURS	ACTIVITY
	<i>Days</i>	<i>Gm.</i>				<i>Per cent</i>
No. 9	137	286	97	1½	698	8
	151	302	111	40	744	12
No. 7	113	354	84	4½	836	11
	134	400	105	40	869	6
No. 5	137	314	83	3½	767	9
	156	336	102	40	726	9
No. 3	114	296	78	1½	833	11
	128	320	92	40	774	18

The average heat production of each rat during the first three consecutive low periods immediately after exercise (italicized in table 1) is recorded in italics in table 2. The heat production of each of the same rats measured 40 hours after exercise at a date 3 weeks after the special measurement is also shown in table 2. At this time the rats had exercised for about 100 days. In an attempt to eliminate, in so far as possible, the effect of the differences in weight of each rat between the time of the first and the second metabolism observation reported in table 2, the heat production in each

case is expressed per square meter of body surface. Comparison of the two observations on each rat shows that the metabolism of rat 9 was lower $1\frac{3}{4}$ hours after exercise than it was in the subsequent measurement 40 hours after exercise. That of rat 7 was not much higher $4\frac{1}{4}$ hours after exercise than it was 3 weeks later, 40 hours after exercise. That of rat 5 was 6 per cent higher $3\frac{1}{4}$ hours after exercise and that of rat 3 was 8 per cent higher $1\frac{3}{4}$ hours after exercise than in the subsequent observations 40 hours after exercise. Moreover, the metabolism of rat 3 was not much lower in the fourth and fifth hours after exercise (table 1). Hence, although the metabolism of these rats reached a plateau in from 2 to 4 hours after exercise, it was in at least two instances from 6 to 8 per cent above the basal level. With rats forced to run 440 meters per hour for 2 hours or about one-third the hourly speed of our exercised rats, v. Árvay and Verzár ('31) noted that the metabolism was high immediately after work and decreased continually until the third half-hour after exercise, when it reached a normal level. The metabolism of our rats that ran at a faster rate for a longer period did not reach a normal level by the third half-hour.

THE AFTER-EFFECT OF EXERCISE UPON RECTAL TEMPERATURE

The marked effect of muscular exercise upon human body functions and the return of these functions to normal after having been affected by exercise have been carefully studied by H. M. Smith ('22). After severe grade walking indications were found of a rapid rise in rectal temperature and an equally rapid fall. The technic of rectal temperature determinations on rats as described in a previous communication (Benedict et al., '32) has been satisfactory. Hence as a part of our study of the immediate after-effect of exercise upon the rat, observations of the rectal temperature were included. Nine male rats were measured that had exercised approximately 65 days and, to study the normal daily rhythm of the body temperature of the non-exercised rat, nine littermate controls were also measured at three different times of day.

The rectal temperatures of the exercised rats were measured before work (8.30 to 9 A.M.), immediately after the rats had run continuously for 4 hours (5486 meters), and thereafter at intervals of 20 minutes for 85 minutes. These measurements were made on three different days at weekly intervals. The results of a typical day alone (average age of rats, 100 days) are presented in table 3.

The rectal temperatures before exercise varied only from 37.1° (rat 5) to 37.9°C. (rats 3 and 7). Although it is debatable whether one should average values for only nine rats,

TABLE 3
Rectal temperatures of rats before and after exercise

RAT NO.	BEFORE EXERCISE (8.30 A.M.)	AFTER EXERCISE ¹ (12.30 TO 2.00 P.M.)				
		0 minute	20 minutes	40 minutes	60 minutes	85 minutes
	°C.	°C.	°C.	°C.	°C.	°C.
1	37.4	39.3	38.2	38.1	37.8	37.7
2	37.3	38.9	38.3	38.6	38.2	37.4
3	37.9	39.2	38.8	37.2	39.1	37.6
4	37.7	39.3	38.2	37.8	37.6	37.6
5	37.1	38.3	37.9	37.4	37.3	37.7
6	37.2	38.9	37.7	37.3	37.0	37.1
7	37.9	39.2	38.8	38.8	38.4	38.1
8	37.2	39.2	38.3	37.8	37.6	37.6
9	37.6	38.4	38.2	37.8	37.7	37.7
Average	37.5	39.0	38.3	37.9	37.9	37.6

¹ After rats had been running 4 hours (5486 meters).

the uniformity in the results is such that the average value of 37.5° may be accepted as representing with reasonable accuracy the pre-work level. Immediately after exercise the temperatures averaged 39.0°, i.e., 1.5° above the pre-work level. Twenty minutes later the temperatures had fallen to an average of 38.3° and in another 20 minutes to 37.9°, at which level they remained essentially constant for the remainder of the time. Occasionally an aberrant value appears, such as the observed temperature of rat 3 (39.1°) at the 60-minute period, which was nearly 2° above the preceding value (37.2°) at the 40-minute period. This rat struggled,

however, during the measurement at the 60-minute interval. The observed rectal temperatures after exercise did not actually reach the pre-work level, but at the end of 85 minutes they were on the average only one-tenth of a degree above the pre-work values.

The measurements before exercise may not, however, be accepted uncritically as the baseline. They were made at 8.30 A.M., 4 hours before the next determinations at 12.30 P.M.

TABLE 4
Daily rhythm of rectal temperatures of non-exercised rats

RAT 10	RAT 11	RAT 12	RAT 13	RAT 14	RAT 15	RAT 16	RAT 17	RAT 18	GROUP AVERAGE
°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Morning ¹									
37.2	37.9	37.6	37.6	37.8	37.4	37.6	36.6	37.4	
37.3	38.0	36.9	37.6	37.6	37.7	37.4	36.9	37.4	
37.3	37.2	37.0	37.2	37.4	38.6	37.0	36.7	37.2	
37.1	38.1	37.0	37.4	37.8	38.0	37.4	37.4	37.6	
37.2	37.8	37.1	37.5	37.7	37.9	37.4	36.9	37.4	37.4
Noon ¹									
37.6	37.9	36.8	37.6	38.0	37.7	37.1	37.3	37.4	
37.2	37.9	37.2	37.7	37.3	37.4	37.4	36.9	37.7	
37.4	37.4	37.0	37.8	37.9	37.6	37.4	37.0	37.6	
37.7	37.6	37.2	37.0	37.6	37.3	37.3	37.3	37.7	
37.5	37.7	37.1	37.5	37.7	37.5	37.3	37.1	37.6	37.4
Evening ¹									
37.3	38.6	37.9	37.0	37.7	38.6	37.4	38.1	38.2	37.9

¹ 9.00 A.M.; 12.00 noon; 6.00 P.M.

immediately after exercise. Hence, if a diurnal rhythm existed, it would appear here. To secure information on any possible diurnal variation in body temperature, control rats (table 4) were measured on 4 different days at 9 A.M. and 12 noon, and on 1 day at 6 P.M. These rats engaged only in spontaneous cage activity and were fairly quiet during the day. Innumerable temperature measurements have already been made upon the normal rat, but the values in table 4 have special interest, since they indicate the rectal tempera-

ture at definite times throughout the day. In general, the values for the individual animals agree well. In the morning the variation in the rectal temperatures of any one rat on the 4 different days was frequently not more than 0.4°C . Rats 11, 15, and 17, however, showed differences of not far from 0.9°C . The average morning temperatures of the several rats on the 4 days differ only by 1.0°C . In the noon series the differences between determinations on the same animal on different days was usually less than in the morning series. Only rats 13 and 14 showed a variation of 0.7° or over in the four measurements. The 4-day noon averages for the various animals agree more closely than the morning averages, differing only by 0.6°C . The greater uniformity is undoubtedly a reflection of the greater degree of repose at midday. In the one series of measurements at 6 P.M. there was a variation of 1.6°C . in the values. The average evening temperature for the group (37.9°) is somewhat higher than the group average at noon (37.4°). Thus the rats had a more uniform rectal temperature when in greatest repose, whereas with probable differences in activity, even only cage activity, they showed greater differences in body temperature. These differences in man would represent an actually febrile state. Judging from our noon series, however, we can assume that the average rectal temperature of the normal resting albino rat at midday is close to 37.4°C .

The rectal temperatures of the exercised rats before work at 8.30 A.M. averaged 37.5° (table 3); immediately after work at noon, 39.0° ; and from 40 to 85 minutes later, 37.9°C . The rectal temperatures of the non-exercised animals averaged 37.4° at 9 A.M. and again at noon. Hence the high temperatures of the exercised rats immediately after exercise at noon cannot be attributed to a diurnal rhythm but must have been an after-effect of the exercise.

THE EFFECT OF PROLONGED MUSCULAR EXERCISE UPON BASAL METABOLISM

Two series of experiments were conducted to determine the effect of prolonged muscular activity upon basal metabolism. In both series the exercised rats were forced to run about 548 meters daily when exercise was initiated. During the first 2 weeks the distance run was gradually increased until the rats in the first series ran 5486 meters daily (table 5) and those in the second series, 1829 meters daily (table 6). The metabolism of these exercised rats was measured several times between the ages of 35 and 156 days, during which time their body weights naturally increased rapidly. Littermates were selected for controls, since our experience has been that the metabolism of littermates is usually in close agreement.⁵ The moderately exercised rats and their littermate controls were fed the basal ration ad libitum and from 200 to 250 mg. of yeast daily. The severely exercised rats (nos. 1 to 9) were given the basal ration ad libitum and 600 mg. of yeast daily; some of their littermate controls (nos. 10 to 14) also received an unlimited basal ration and from 200 to 400 mg. of yeast daily, and others (nos. 15 to 18) were given a restricted basal ration and 600 mg. of yeast daily. These two methods of feeding were employed in an attempt to maintain the rate of growth of the controls at a rate comparable to that of the exercised rats. The controls were not exercised but were permitted the cage activity normal for a rat living alone. For 24 hours prior to the metabolism measurements the rats lived in a special cage (Benedict and Riddle, '29) at a known and constant environmental temperature of 25° or 28°C. After a 1-hour preliminary period of adjustment to the experimental conditions, the time required for each rat to absorb a known volume of oxygen was determined. Three or more consecutive periods, circa 50 to 100 minutes long, were carried out between 10 A.M. and 4 P.M.

⁵Greenman and Duhring ('31) state that animals from the same litter are more nearly alike than others of the same age.

Since in all our experiments exercise was initiated when the rats were about 30 days old, the factor of growth or advancing age undoubtedly enters into this study. Likewise, it is conceivable that the effect of muscular activity may be more pronounced in the young growing rat than in the same animal when full grown. The effect may also be more pronounced early in the period of training. Another point to be considered is that, although littermates were selected for controls, thus ruling out the influence of difference in age in the different age groups, the attempt to feed the control animals so that their weights would be the same as those of the exercised rats was only partly successful in the case of the first series (severe exercise). As will be seen from analysis of the data in table 5, the metabolism of the controls fed restricted rations was somewhat lower (at all the different ages) than that of the controls on full rations. Hence, the average values reported in table 5 for the controls are somewhat lower than they would be had all the control rats been on full rations. Still another point to be held in mind is that the control rats were not wholly inactive. How active they were is not known. Moreover, although the distance traveled by the exercised rats was ascertained, this distance does not represent a quantitative estimate of the amount of work done. The rats could not cling to the sides of the cage and be carried around by mechanical action, but they might have slipped and rolled inside the cage.

To eliminate, in so far as possible, the effect of differences in weight, investigators have commonly expressed the metabolism in terms of the heat production per square meter of body surface. In order to present our data in a form comparable to that employed by earlier investigators and pending a future calculation on some other basis, we have referred the heat production of our rats in tables 5 and 6 to the body surface. Our moderately exercised rats weighed essentially the same as their controls. Likewise, in the severe exercise series the weights of the controls themselves were much the same in any given age group. Hence, we have also reported

TABLE 5

Effect of prolonged, severe (5486 meters per day) exercise upon the basal metabolism of the growing male rat (measured 24 hours after food, 40 hours after exercise, and at 30°C.)

EXERCISED RATS					NON-EXERCISED RATS			
Rat no.	Age	Weight	Days of exercise	Heat production per sq.m. per 24 hours	Rat no.	Age	Weight	Heat production per sq.m. per 24 hours
	<i>Days</i>	<i>Gm.</i>		<i>Cal.</i>		<i>Days</i>	<i>Gm.</i>	<i>Cal.</i>
1	48	95	18	947	10	48	94	888
2	65	132	25	886 ¹	11	48	97	828
3	51	104	15	930	12	51	87	838
4	65	115	25	1000	13	62	100	852
5	67	118	13	913	15	43	99	754
6	62	115	22	940	16	51	86	831
7	43	107	14	985	17	62	111	838
8	56	99	18	908	Aver.	52	96	833
9	65	119	25	900				
Aver.	58	112	19	934				
2	86	188	46	912	14	72	123	960 ¹
1	76	197	46	1062	10	76	171	832
3	72	175	36	944	11	76	171	835
4	93	180	53	773 ¹	13	93	172	874 ¹
5	88	182	34	859	18	76	158	723
6	93	187	53	822 ¹	15	76	167	739
7	83	274	54	876	16	79	167	824
8	79	187	41	891	17	88	161	783
9	88	182	48	814	Aver.	80	161	821
Aver.	84	195	46	884				
1	99	240	69	979	11	99	238	901 ¹
3	91	249	55	870	10	99	233	843
2	114	247	74	809	12	121	293	818
4	121	237	81	782	14	121	233	807
5	109	255	55	845	13	121	238	896 ¹
6	114	240	74	866	18	99	232	819 ¹
8	121	284	83	755	16	121	285	779
9	114	255	74	782 ¹	17	121	247	736 ¹
Aver.	110	251	71	836	Aver.	113	250	825
1	132	308	102	768	10	132	298	784
3	128	320	92	774 ¹	12	142	328	691
4	151	292	111	740	13	142	279	716
5	156	336	102	726	18	134	296	738
6	142	276	102	758	15	141	352	645
2	156	290	116	773 ¹	17	151	302	747
7	148	410	119	818	Aver.	140	309	720
9	151	302	111	744				
Aver.	146	317	107	763				

¹ Rats were active from 15 to 20 per cent of the period of measurement; activity was less than 15 per cent for values not marked.

TABLE 6

Effect of prolonged, moderate (1829 meters per day) exercise upon the basal metabolism of the growing male rat (measured 24 hours after food, 40 hours after exercise, and at 30°C.)

EXERCISED RATS					NON-EXERCISED RATS			
Rat no.	Age	Weight	Days of exercise	Heat production per sq.m. per 24 hours	Rat no.	Age	Weight	Heat production per sq.m. per 24 hours
	<i>Days</i>	<i>Gm.</i>		<i>Cal.</i>		<i>Days</i>	<i>Gm.</i>	<i>Cal.</i>
19	44	94	14	856	28	44	96	916
20	45	87	14	749	29	45	98	798
21	42	98	12	803	30	42	88	822
22	35	67	8	847	31	35	74	763
23	42	70	6	813	32	42	77	800
24	44	84	14	777	33	44	80	899
25	42	74	6	831	Aver.	42	86	833
26	42	72	6	810				
27	35	70	8	781				
Aver.	41	80	10	807				
19	60	132	30	903	28	60	138	909
20	64	134	33	761	29	64	134	727
21	58	139	28	869	30	58	130	906 ¹
22	56	104	29	861	31	56	113	746
23	57	102	21	784	32	57	102	834
24	70	145	40	681	Aver.	59	123	824
25	57	91	21	848				
26	57	100	21	806				
27	56	113	29	812				
Aver.	59	118	28	814	30	84	194	990 ¹
21	86	190	56	807	31	75	160	713
22	75	148	48	835	32	76	141	915 ¹
23	76	144	40	896	Aver.	78	165	873
25	76	149	40	891				
26	76	141	40	874 ¹				
27	84	154	57	816	28	88	189	840 ¹
Aver.	79	154	47	853	29	94	188	763
19	93	191	63	954	Aver.	91	189	802
20	99	190	68	748				
23	99	178	63	872				
24	91	186	61	754				
25	97	193	61	855	28	123	230	611
26	99	176	63	899	29	133	236	713
Aver.	96	186	63	847	30	114	234	815
20	133	229	102	804	31	131	236	724 ¹
21	114	234	84	783	32	134	224	851
22	131	228	104	877	Aver.	127	232	743
23	132	232	96	860 ¹				
24	128	244	98	696				
25	132	251	96	881				
26	139	219	103	870 ¹				
27	131	239	104	980 ¹				
Aver.	130	235	98	844				

¹ Rats were active from 15 to 20 per cent of the period of measurement; activity was less than 15 per cent for values not marked.

the basal metabolism of each age group in both series on the basis of the average heat production per 200 gm. of body weight per 24 hours (table 7).

The metabolism of the severely exercised rats and their controls (table 5), both per square meter of body surface and per 200 gm. of body weight decreased, on the average, with age. On the contrary, the metabolism of the moderately exercised rats (table 6) per unit of surface area did not decrease with age. Per unit of weight, however, the metabolism of the moderately exercised rats did show the characteristic

TABLE 7
Average heat production per 200 gm. of body weight per 24 hours of exercised rats and their controls

EXERCISED RATS				CONTROLS		
Distance run daily	Average age	Average weight	Calories per 200 gm. per 24 hours	Average age	Average weight	Calories per 200 gm. per 24 hours
<i>Meters</i>	<i>Days</i>	<i>Gm.</i>		<i>Days</i>	<i>Gm.</i>	
5486	58	112	35.5	52	96	33.1
5486	84	195	27.9	80	161	27.6
5486	110	251	24.1	113	250	23.9
5486	146	317	20.4	140	309	19.4
1829	41	80	34.3	42	86	34.5
1829	59	118	30.4	59	123	30.2
1829	79	154	29.1	78	165	29.0
1829	96	186	27.1	91	189	25.5
1829	130	235	24.9	127	232	22.0

decrease with advancing age and increasing weight. The controls for this series showed a lower metabolism with advancing age on both bases of calculation.

On the body-surface basis the average heat production of the severely exercised rats at an average age of 58 days was 12 per cent higher than the average for their littermate controls, and at 84 days of age it was 8 per cent higher. At 110 days and 146 days it was still somewhat higher than that of the controls, but not significantly higher. Indeed, at 146 days the heat production of both the severely exercised and the control rats approached the average metabolism, 708

calories, reported previously (Benedict, Horst and Mendel, '32) for adult male rats weighing from 300 to 400 gm. On the body weight basis the average metabolism of the severely exercised rats in the several age groups was not appreciably higher than the average metabolism of their controls.

The average heat production of the moderately exercised rats was almost the same per unit of surface area at 41 days of age, with 1 or 2 weeks of previous exercise, as at 59 days of age, after 3 to 4 weeks of exercise. Likewise, the average metabolism was essentially the same as that of the littermate controls at these ages. It is possible that the exercised rats were quiet when not forced to run and that the spontaneous activity (not recorded) of the caged controls throughout the night was so great that in the end the exercise of the animals in the two groups was about the same. It is also possible that the exercised rats did not receive sufficient vitamin B. Initially they were given not much more than 200 mg. of dry brewers' yeast daily. Keith and Mitchell ('23) concluded that forced running (about 3 miles a day) did not clearly affect the rat's requirement for vitamin B. More recently, however, Cowgill, Rosenberg, and Rogoff ('31) have shown that in the dog the time required to deplete the vitamin B reserve is decreased by exercise. At 79 days the moderately exercised rats had again much the same average metabolism per square meter of body surface as their controls, but at 96 and 130 days their metabolism on this basis was higher than that of the controls. On the body weight basis the moderately exercised rats had a heat production averaging essentially the same as that of their controls.

If one were to consider only the heat production of the severely exercised rats and their controls on the basis of per unit of surface area, one could conclude that the severe exercise (although the exercise had ceased 40 hours before the metabolism was measured) increased the basal metabolism of the rats in the growing stage, but that when the adult weight (about 300 gm.) was reached, the severely exercised rats had a metabolism typical of the normal non-exercised

rat. Against such a conclusion, however, is the fact that per unit of body weight the metabolism of the severely exercised rats was much the same as that of their controls (especially if one considers only the controls on full feed) and that on both bases of calculation the metabolism of the moderately exercised rats was essentially the same as that of their controls, at all ages. Since the controls for the moderately exercised rats were more ideal for comparison, being nearly of the same weights as the exercised rats, it seems reasonable to conclude that continued, forced running amounting to at least 1829 meters and probably to 5486 meters daily has no influence on the basal metabolism of the rat.

This finding is difficult to interpret, since one would expect that prolonged exercise would cause a pronounced alteration in the composition of the body (less deposition of fatty tissue) and that the hypertrophy of protoplasmic tissue, particularly muscle tissue, would result in a greater cell activity and hence an increased metabolism. This would be in harmony with the general view that athletically trained individuals have a high metabolism. This last point, however, is justly debatable. Benedict and Smith ('15) considered that the athletes studied by Smith at Syracuse University had a metabolism somewhat higher than normal. In a subsequent recalculation of these values, Harris and Benedict ('19) decided the difference was not so great as had previously been estimated. Schneider and his associates at Wesleyan University ('27, '31) found that in some instances the metabolism of human subjects was lowered by exercise, in others increased, and in others there was no change. It is a question whether in the cases mentioned the men were given proportionately the same amount of exercise that our rats were given. The exercise of man is usually maintained only during a relatively short time, whereas our rats ran for many hours daily. Hence the exercise entered as a larger factor in the case of our rats than in either of the cases cited for man.

The contradictory results shown by the heat values (per unit of body surface and of body weight) of our severely exercised

rats are undoubtedly ascribable to the fact that the controls used for comparison were not of the same weight as the exercised rats. This was a defect in our experimental technic. It also accentuates the chaotic state existing at the present day in the methods employed in comparing the metabolism of animals of different sizes, even of the same species, and especially challenges the infallibility of the surface-area comparison. Obviously, in any further experiments where the effect upon the basal metabolism of some superimposed factor is to be studied, the control animals must be of the same age and weight throughout the entire period of comparison, no matter what experimental difficulties are entailed in the securing of such controls.

RELATION OF FOOD CONSUMPTION TO SEVERE EXERCISE OF GROWING RATS

The food consumed by the severely exercised and by the control rats during the experimental period of about 120 days is recorded in table 8, expressed as the average daily food intake of each rat. The relation of food consumption to increment in weight is also shown. Since the food was essentially water-free and compounded so that 1 gm. had a caloric value of about 5 calories, we can deal directly with the relative weights of food consumed. Per gram of gain in weight the exercised rats consumed more food than the controls, 5.1 as compared with 4.3 gm. The caloric intake was thus about 26 calories per gram of gain in weight for the exercised rats and 22 calories for the controls. The total daily food intake of the exercised rats averaged 11.3 gm. and that of the controls, 9.1 gm. The exercised rats therefore consumed daily 2.2 gm. or about 24 per cent more than did the non-exercised rats. Since both groups started at the same weight (about 60 gm.) and ended at approximately the same weight (about 300 gm.), one might infer that the muscular work of the exercised rats raised the metabolism only approximately 24 per cent, that is, in proportion to the increase in food consumption. In any comparison of the food intake

with gain in weight from the energetic standpoint, however, one must always realize that there is a large element of uncertainty with regard to the normal energy requirements of the animals when not exercising. In analyzing cases like these the old, classic method of Rubner of determining the total metabolism throughout 24 hours would have been highly

TABLE 8

Relation of severe exercise (5486 meters daily) to food consumption of growing male rats

RAT NO. ¹	DURATION OF EXPERIMENT	BODY WEIGHT			FOOD INTAKE	
		Initial	Final	Average daily gain	Average per day	Per gram gain in weight
	<i>Days</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>
Exercised						
1	110	58	309	2.3	10.7	4.7
2	126	61	286	1.8	10.8	6.1
3	115	70	339	2.3	11.6	5.0
5	128	60	344	2.1	10.8	5.0
7	133	70	427	2.7	13.7	5.1
8	115	51	316	2.3	10.9	4.7
9	126	60	312	2.0	10.7	5.4
Average					11.3	5.1
Controls						
10	121	62	305	2.0	9.9	4.9
12	122	56	324	2.2	10.0	4.5
13	133	63	316	1.9	9.1	4.7
15 ²	134	68	381	2.3	8.9	3.8
16 ²	123	58	327	2.2	8.7	4.0
17 ²	134	67	336	2.0	8.8	4.4
18 ²	121	50	303	2.1	8.6	4.1
Average					9.1	4.3

¹ Rats lived at 25°C.

² Rate of growth controlled by limiting food consumed.

successful. Our complete lack of knowledge of the metabolism of these rats, not under basal conditions, but while resting or at least while not forcibly exercised, complicates the situation enormously. Measurement of the metabolism in a calorimeter, where the rats would be allowed normal, free activity as compared with the forced activity in the exercise chamber, would have solved the problem.

It is inconceivable, however, that the severe exercise (5486 meters daily) increased the metabolism only 24 per cent, unless the spontaneous activity of the controls was nearly the same as the forced exercise of the experimental rats, under which conditions the difference in energy consumption would represent the true difference in heat production. This brings out strikingly the difficulty of analyzing food intake in terms of energy, especially where there is a possibility that the superimposed factor of exercise may have altered the body composition. It is logical to consider the food intake from the standpoint of calories, but it is not correct to consider the increase in an animal's weight from the energy standpoint. In such a consideration one assumes that material of the same composition and hence of the same energy value was added to the body weight throughout the entire time. However, there may have been a deposition of fat and a compensating loss of water, which would alter the energy value of the body material. It is perhaps surprising that the exercised rats did not eat considerably more than the controls. We have no knowledge of the actual distance covered by the controls. They were allowed 'cage activity,' but we do not know to what extent they roamed around their cages at night. On the other hand, we also do not know how quiet, relatively, the exercised rats were at night following their severe exercise during the day. We might fairly assume that there was a compensation in activity and that the exercised rats did not travel around as much at night as did the controls.

THE BODY COMPOSITION

The idea that severe, long-continued exercise will alter the deposition of fatty tissue and possibly the transport of water or the variation of water content is not new. Reed, Yamaguchi, Anderson, and Mendel ('30) found that exercised rats deposited less fat than non-exercised rats. Variations in body composition have also been cited to account for differences in metabolism. In general, the bodies of our severely exercised rats (5486 meters daily) were 3 per cent richer in water

than the bodies of the controls and consequently contained a lower percentage of dry matter (table 9). On the basis of fresh weight the bodies of the exercised rats contained, on the average, 3.5 per cent less fat, 0.35 per cent more ash, and 0.06 per cent more nitrogen than those of the controls. On the basis of dry matter the exercised rats had a higher ash content than the controls, the difference amounting to 1.58 per cent. The average nitrogen content was 0.78 per cent higher in the exercised rats than in the controls. However, some rats in both the exercised and the control groups showed high nitrogen percentages. For example, rat 1 (exercised) had a nitrogen content of 10.25 per cent and rat 16 (not exercised) of 10.13 per cent. These two rats had the lowest dry weights and the lowest fat percentages of all the animals in both groups. On the dry basis the average fat content of the exercised rats was 6.4 per cent lower than that of the controls. The range in the fat content on the dry basis was greater in the controls than in the exercised animals. The percentage of residual protoplasmic matter, devoid of extraneous components, was apparently constant so far as indicated by the nitrogen content. Thus the percentage of nitrogen in the moisture, fat, and ash-free tissue averaged 16.4 for both groups of rats.

In general the influence of prolonged severe exercise was to raise the water content and lower the fat content of the body. Of the dry matter remaining, the ash content was higher, the nitrogen content higher, and the fat content lower than in the controls. Aside from the high percentage of ash noted with rat 1, no other striking changes in the body composition of the exercised rats were apparent. The heat production was in no way related to the ash content. The heat production singularly enough had no direct relationship to the nitrogen content. To be sure, control rats 15 and 12 with the lowest heat production also had the lowest nitrogen content. On the other hand, control rat 10 with the highest metabolism had a medium nitrogen content, and exercised rats 8, 9, and 1 with heat values somewhat lower than that

TABLE 9
The influence of prolonged severe exercise upon the body consumption of male rats

RAT NO.	DURATION OF EXPERIMENT	FRESH WEIGHT	DRY WEIGHT	PER CENT OF SOLIDS	FRESH WEIGHT				DRY WEIGHT				PER CENT OF NITROGEN MOISTURE-ASH FAT-FREE
					Moisture	Fat	Nitrogen	Ash	Fat	Nitrogen	Ash		
	Days	Gm.	Gm.		Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	
Exercised	1	309	96	31.1	68.9	6.7	3.19	3.78	21.5	10.25	12.17	15.5	
	2	286	105	36.7	63.3	13.7	3.18	3.55	37.0	8.66	9.67	16.2	
	3	339	126	37.2	62.8	14.8	3.10	3.45	40.0	8.33	9.27	16.4	
	5	344	129	37.5	62.5	15.4	3.09	3.51	41.1	8.25	9.35	16.7	
	7	427	152	35.6	64.4	13.5	3.12	3.24	37.9	8.76	9.11	16.6	
	8	316	114	36.1	63.9	13.8	3.39	3.39	38.4	9.40	9.40	18.0	
	9	312	114	36.5	63.5	14.2	2.94	3.41	38.8	8.05	9.34	15.5	
	Average			35.8	64.2	13.2	3.14	3.48	36.4	8.81	9.76	16.4	
	Controls												
10	122	305	114	37.4	62.6	15.6	3.07	3.48	41.6	8.22	9.31	16.7	
12	123	324	126	38.9	61.1	17.5	2.86	3.24	44.8	7.36	8.33	15.7	
13	134	316	123	38.9	61.1	16.0	3.21	3.25	41.2	8.25	8.35	16.4	
15 ¹	135	381	160	42.0	58.0	22.7	3.04	2.93	53.8	7.23	6.98	18.4	
16 ¹	123	327	109	33.3	66.7	9.8	3.37	3.16	29.4	10.13	9.49	16.6	
17 ¹	134	336	133	39.6	60.4	16.9	3.03	2.83	42.8	7.64	7.15	15.3	
18 ¹	122	303	121	39.9	60.1	18.2	2.95	3.05	45.7	7.40	7.64	15.9	
				38.6	61.4	16.7	3.08	3.13	42.8	8.03	8.18	16.4	

¹ Rate of growth controlled by limiting food consumed.

of rat 10 had higher nitrogen contents. There was also no direct relationship between the basal metabolism and the fat content. In the exercise series the lowest heat production was observed on rat 5, which had the highest fat content of any of the rats in this group. But rat 7 with the highest metabolism had a medium fat content and rat 1 with an intermediate metabolism had a low fat content. In the control series rat 15 with the lowest metabolism had the highest fat content and rat 16 with a relatively high metabolism had a low fat content, but rat 10, also with a high metabolism, had a medium fat content. From these data, on the whole, no real difference in metabolism is ascribable to variations in fat content.

The metabolism of our controls was lower and the fat content higher than the values reported by Brendle ('31) for rats of the same size and sex. This comparison supports Brendle's conclusion that the metabolism is lower with a higher percentage of fat. It is debatable, however, whether metabolism measurements as short as 10 minutes are justifiable. Since only 10-minute periods were used by Brendle and his averages are based on periods with greatest repose, one would expect his values in these selected short periods to be even lower than those in our experiments in which the metabolism was measured during at least 2 or 3 hours (three consecutive periods). Another fact to be considered is that our exercised rats had nearly 4 per cent less fat than our controls and yet their metabolism was approximately the same.

SUMMARY

The oxygen consumption (at 30°C. and 24 hours after food) of four male rats was high immediately after exercise, and 1 hour after exercise it was still from 8 to 10 per cent above subsequent low periods. In from 2 to 4 hours after exercise the metabolism reached a plateau that was approximately basal for two of the rats but still from 6 to 8 per cent above basal for the other two rats.

The average rectal temperature of nine male rats after exercise (39.0°) was 1.5° higher than the pre-work level ($37.5^{\circ}\text{C}.$). During the first 40 minutes after exercise the temperatures fell rapidly and during the next 45 minutes remained essentially constant at a level, on the average, from 0.1° to $0.4^{\circ}\text{C}.$ above the pre-work values. The average rectal temperature of non-exercised rats at noon was the same as in the morning, $37.4^{\circ}\text{C}.$

Prolonged severe exercise (5486 meters daily) increased the basal heat production per square meter of body surface (measured 40 hours after exercise) only of growing male rats, but not of adult rats. Per unit of body weight, on the contrary, the metabolism of the exercised rats was much the same as that of their littermate controls, both during growth and during adult life. The picture is not clear because the controls were not of the same body weights as the exercised rats and were fed somewhat differently.

Prolonged moderate exercise (1829 meters daily) did not modify the basal metabolism of the growing and adult male rats, either per unit of body weight or per unit of body surface. In this study the littermate controls were fed exactly the same as the exercised rats and weighed approximately the same.

The basal metabolism of both groups of exercised rats and of the controls decreased with advancing age.

The severely exercised rats (5486 meters daily for 120 days) consumed only about 24 per cent more food than the non-exercised rats. On the basis of fresh weight the bodies of the exercised rats contained 3.5 per cent less fat and 3 per cent more water than the bodies of the non-exercised rats. On the dry basis the exercised rats had an ash content 1.58 per cent higher, a nitrogen content 0.78 per cent higher, and a fat content 6.4 per cent lower, on the average, than the non-exercised rats. Variations in basal metabolism were not directly related to differences in ash, nitrogen, and fat content.

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THE EFFECTS OF SOME EXTERNAL FACTORS UPON THE METABOLISM OF THE RAT¹

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ONE FIGURE

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In our observations on rats covering more than 3 years of research, data have been obtained concerning the zone of thermic neutrality for basal metabolism measurements, the reaction of the adult and the young, growing rat to low environmental temperatures as expressed by the percentage increase in metabolism per degree lowering of temperature, the influence of time of day, and the effects of light and dark upon the basal metabolism.

Male and female albino rats from the Osborne and Mendel colony were used in all experiments except the second series of light and dark studies, when female rats from the colony of the Bussey Institution of Harvard University were kindly furnished by Prof. W. E. Castle. Oxygen consumption was usually measured with a multiple-chamber respiration apparatus (Benedict, '30). On one series (experiments on the influence of light and dark), carbon dioxide elimination was determined with a simple form of respiration chamber which has already been described by Benedict and Petřík ('30). The rats were usually maintained at an environmental temperature of 23° to 25°C. When the metabolism was to be measured

¹ The data in this paper are taken from a dissertation presented by Kathryn Horst in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Yale University, 1931.

at thermic neutrality, they lived in a glass cage at 26° or 28°C. for 24 hours before the metabolism experiments. In studies on the effect of a lower temperature, the rats lived at that temperature during the 24-hour preparatory period, i.e., rats 23 to 34 lived at 16° and rats 35 to 38 at 20°C. Water was always available during the fasting periods. In experiments not concerned with diurnal changes, metabolism was measured between 10 A.M. and 4 P.M. The rats were studied individually in the respiration chamber, except in the second light and dark series, when two rats huddling were measured. The heat production was calculated as explained in previous papers (Horst, Mendel and Benedict, '30, '32, '34).

DIURNAL VARIATIONS IN METABOLISM

Rubner has emphasized that metabolism measurements should cover 24 hours and that unless 24-hour measurements are made, the data are not suitable for the study of his surface-area law. Slonaker ('24) and Richter ('27), however, have shown that the activity of male and female rats differs from day to day. Hence the comparison of two rats on the basis of 24-hour measurements will obviously always show a greater degree of variability in metabolism than the comparison of two rats studied in shorter periods under complete repose. Stier ('30) in 12-hour experiments on mice (40 days old) found variations in the type of activity in different experiments. Rubner worked with fasting animals in his 24-hour measurements of basal metabolism. Our observations on the diurnal variation in the rat's metabolism, however, were incidental to a study of the effect of food upon metabolism. Four rats (table 1) were measured in consecutive periods from 2 A.M. to 10 P.M. Prior to these observations food had been withheld for 9 hours, was subsequently accessible for 4½ hours, and then was not available for 2 hours. The metabolism of each rat was high at about 2 A.M., or 2 hours after food, but tended to decrease thereafter, and minimum values were observed between 10 A.M. and 4 P.M. Unquestionably, the high metabolism in the first periods of

TABLE 1

The metabolism of rats at 28°C., in the first 20 hours after withdrawal of food

RAT NO., SEX, AGE AND WEIGHT ¹	PERIOD OF OBSERVATION		HOURS AFTER FOOD ²	ACTIVITY	O ₂ CONSUMED	
	Duration	Minutes			Total per period	Per 200 gm. ³ per minute
No. 1, ♀	2.17 A.M.—3.43 A.M.	86	2	30	322	3.74
165 days	3.43 A.M.—5.00 A.M.	77	3½	40	331	4.26
215 gm.	5.00 A.M.—6.11 A.M.	71	5	45	328	4.57
202 gm.	6.11 A.M.—7.41 A.M.	90	6	13	335	3.69
	7.41 A.M.—9.17 A.M.	96	7½	17	332	3.42
	9.17 A.M.—10.58 A.M.	101	9	10	331	3.24
	10.58 A.M.—12.36 P.M.	98	10½	9	330	3.34
	12.36 P.M.—2.25 P.M.	109	12½	14	336	3.05
	2.25 P.M.—4.04 P.M.	99	14	15	328	3.28
	4.04 P.M.—5.38 P.M.	94	16	18	321	3.38
	5.38 P.M.—6.56 P.M.	78	17½	35	321	4.07
	6.56 P.M.—8.18 P.M.	82	18½	23	329	3.97
	8.18 P.M.—9.42 P.M.	84	20	13	328	3.87
Total		19 hr. 25 min.			4272	
No. 2, ♀	2.15 A.M.—3.28 A.M.	73	2	34	327	3.88
157 days	3.28 A.M.—4.40 A.M.	72	3	36	325	3.91
247 gm.	4.40 A.M.—5.59 A.M.	79	4½	27	328	3.59
231 gm.	5.59 A.M.—7.28 A.M.	89	5½	7	354	3.44
	7.28 A.M.—8.48 A.M.	80	7	11	310	3.36
	8.48 A.M.—10.12 A.M.	84	8½	5	330	3.41
	10.12 A.M.—11.50 A.M.	98	10	6	331	2.92
	11.50 A.M.—1.34 P.M.	104	11½	11	334	2.78
	1.34 P.M.—3.19 P.M.	105	13½	11	329	2.72
	3.19 P.M.—4.43 P.M.	84	15	33	325	3.35
	4.43 P.M.—6.01 P.M.	78	16½	49	324	3.60
	6.01 P.M.—7.10 P.M.	69	18	30	321	4.03
	7.10 P.M.—8.15 P.M.	65	19	43	331	4.41
	8.15 P.M.—9.24 P.M.	69	20	28	335	4.20
Total		19 hr. 9 min.			4604	
No. 3, ♂	2.12 A.M.—3.36 A.M.	84	2	35	318	5.05
79 days	3.36 A.M.—5.18 A.M.	102	3½	17	324	4.24
157 gm.	5.18 A.M.—6.53 A.M.	95	5	17	332	4.66
150 gm.	6.53 A.M.—8.46 A.M.	113	6½	8	339	4.00
	8.46 A.M.—10.38 A.M.	112	8½	11	330	3.93
	10.38 A.M.—12.27 P.M.	109	10½	16	330	4.04
	12.27 P.M.—2.18 P.M.	111	12	18	337	4.05
	2.18 P.M.—4.04 P.M.	106	14	16	317	3.99
	4.04 P.M.—5.45 P.M.	101	16	23	322	4.25
	5.45 P.M.—7.18 P.M.	93	17½	20	325	4.66
	7.18 P.M.—8.55 P.M.	97	19	30	338	4.65
Total		18 hr. 43 min.			3612	
No. 4, ♂	3.53 A.M.—5.40 A.M.	107	3½	12	342	4.50
76 days	5.40 A.M.—7.29 A.M.	109	5½	7	322	4.16
152 gm.	7.29 A.M.—9.25 A.M.	116	7	11	333	4.04
142 gm.	9.25 A.M.—11.38 A.M.	133	9	6	331	3.50
	11.38 A.M.—1.37 P.M.	119	11½	25	333	3.94
	7.02 P.M.—8.53 P.M.	111	19	22	332	4.21

¹ First weight in each case is weight at start and second is that at end of experiment.² Estimated from time of withdrawal of food to beginning of period of observation; result is expressed to nearest half hour.³ Based on weight of rat at close of experiment.

measurement was in part the result of the after-effect of food but in greater part the result of excessive activity.

To ascertain whether the basal level had been reached in these experiments, the heat production per square meter of body surface per 24 hours has been calculated for each rat from the measured oxygen consumption during three to five consecutive low periods in the middle of the day. These heat values (italicized and entitled 'special measurement') and the values obtained in previous and subsequent determinations 24 hours after food under basal conditions are given in table 2. Comparison of these results shows that, whereas the heat production of rats 2 and 4 in the 'special measure-

TABLE 2

A comparison of the metabolism of rats (at 30°C.) 7 to 10 hours after food with that 24 hours after food

RAT NO, SEX, AND METABOLISM MEASUREMENT	AGE	WEIGHT	PERIOD OF OBSERVATION	HOURS AFTER FOOD	HEAT PRODUCTION PER 24 HOURS	
					Per 200 gm.	Per square meter
	<i>Days</i>	<i>Gm.</i>			<i>Cal.</i>	<i>Cal.</i>
No 1, ♀						
Previous basal	134	196	10 12 A.M.—4.29 P.M.	24½	18.5	590
Previous basal	148	202	9.54 A.M.—3.31 P.M.	24	19.5	629
Special measurement	165	202	9.17 A.M.—4.04 P.M.	9	<i>21.9</i>	<i>705</i>
Subsequent basal	172	202	9.50 A.M.—3.46 P.M.	23	19.1	617
No. 2, ♀						
Previous basal	126	213	10.03 A.M.—3.02 P.M.	24½	21.3	701 ¹
Previous basal	154	234	9.47 A.M.—2.24 P.M.	23	21.0	711
Special measurement	157	231	10.12 A.M.—3.19 P.M.	10	<i>19.0</i>	<i>640</i>
Subsequent basal	164	236	9.53 A.M.—3.19 P.M.	23	17.9	607
No 3, ♂						
Previous basal	72	137	10 47 A.M.—4.48 P.M.	18	27.2	769
Special measurement	79	150	6 53 A.M.—4 04 P.M.	6½	<i>27.1</i>	<i>791</i>
Subsequent basal	86	168	9.56 A.M.—5.04 P.M.	17	25.6	776
No. 4, ♂						
Previous basal	62	108	10 07 A.M.—3.44 P.M.	18½	24.3	636 ¹
Special measurement	76	142	9.25 A.M.—1.37 P.M.	9	<i>25.2</i>	<i>722¹</i>
Subsequent basal	81	158	9 59 A.M.—5 56 P.M.	17	24.3	722

¹ Denotes metabolism with activity in excess of 15 per cent.

ment' reached a basal level in 9 to 10 hours after food, the metabolism of rats 1 and 3 in the 'special measurement' remained somewhat above basal values. Although the exact time when food was eaten is not known, it is probable that at the time of the special measurements our rats had been fasting from 3 to 4 hours longer than is indicated in tables 1 and 2. Furthermore, the rats may have consumed considerable food in the first hour when food was available and none in the last $3\frac{1}{2}$ hours.² In that event rats 2 and 4 would have been without food from 12 to 14 hours when the metabolism (special measurement) reached a basal level. Wesson ('31) and v. Árvay and Verzář ('28) found that the heat production of the rat was at a minimum in 14 to 16 hours after food. Benedict and MacLeod ('29) noted that the metabolism of the rat decreased during the first 17 to 24 hours after food and remained essentially constant thereafter up to the sixty-fourth hour. Moreover, Horst, Mendel, and Benedict ('30) observed respiratory quotients not far from 0.72 in 16 to 23 hours after food. Hence it appears that the ingestion of food does not exert any appreciable influence on the rat's metabolism after 14 to 17 hours.

The total heat production of three of our rats for the 24-hour cycle has been estimated from the data in table 1 by projecting the oxygen value (total per period) observed at about 2 A.M. back to midnight and at 9 or 10 P.M. forward to midnight and calculating the heat production from the total volume of oxygen consumed in 24 hours. On this basis the metabolism per 200 gm. of body weight per 24 hours was 24.8, 24.3, and 29.9 calories for rats 1, 2, and 3, respectively. The heat production of these same rats in short periods at midday under quiet basal conditions averaged only 19.0, 20.1, and 26.4 calories, respectively (table 2). On the basis of body surface the heat values are 799, 819, and 872 calories

² During the $4\frac{1}{2}$ hours when food was accessible, rat 1 consumed 3 gm. of a special dog biscuit and 20 gm. fresh lettuce; rat 2, 7 gm. of the biscuit and 20 gm. fresh lettuce, rat 3, 4 gm. of a synthetic diet, and rat 4, 7 gm. of the same diet. Rats 3 and 4 each ingested 200 mg. dry brewers' yeast 2 hours before oxygen consumption was measured.

(24-hour measurements) and 612, 673, and 773 calories (basal measurements several hours in duration). Thus the metabolism based on 24-hour measurements was from 13 to 31 per cent higher than that calculated from 4- to 6-hour measurements made during midday.

The metabolism of rat 2 (table 1) was 50 per cent higher, that of rat 1 was 24 per cent higher, and that of rat 3 was 13 per cent higher after 4 P.M. (17 to 20 hours after food) than it was during midday (9 to 12 hours after food). This higher metabolism after 4 P.M. cannot be attributed to the after-effect of food. It may be coincident with an increased activity associated with the normal feeding habits of the animals. Food was accessible to these rats (except when fasting for an experiment) at all hours, but fresh food was always placed in the cages between 3 and 5 P.M. Szymanski ('18) found that his rats were usually active less than 25 per cent of the time between 6 A.M. and 4 P.M., whereas from 4 P.M. to 6 A.M. they were active more than 25 per cent of the time. The activity of our rats, especially rats 1 and 2, corresponds to the diurnal variations observed by Szymanski.

Our experimental evidence is so obscured by the uncontrollable muscular activity of the rats that the question as to whether there is a diurnal variation in metabolism irrespective of the activity accompanying the night period remains unsolved. Undoubtedly, throughout the night our rats were subjected to a less degree than during the day to external stimuli of noises around the laboratory. But they were most active at night. It is striking that in the daytime with the various noises in the street and in the building the rats retained as low a metabolism as was found for the most part in these experiments. Perhaps the most important finding in this study is that it is practically out of the question to determine the basal metabolism of rats for comparative purposes before 10 A.M. and after 4 P.M.

METABOLISM IN ABSOLUTE DARKNESS DURING MIDDAY

Earlier observations at Columbia University (Benedict and MacLeod, '29), made during the day, indicated that rats were quieter in a respiration chamber of glass than in complete darkness. Szymanski ('18) found that rats were active for a longer time during the 24-hour cycle when in complete darkness than when exposed to the diurnal variations of light and dark. Moleschott and Fubini (1881) reported carbon-dioxide excretion higher in the light than in the dark. On the other hand, Bergfeld ('31) observed that the metabolism of rats living in darkness was essentially the same as that of animals exposed to various conditions of light. Benedict and Riddle ('29) found indications that light increased the metabolism of ring doves, although their kymograph records gave evidence that the birds were more active in the light than in the dark. Kestner, Johnson and Laubmann ('31) have shown that human metabolism is elevated by exposure to sunlight. It is evident that in a study of the effect of darkness or light upon the metabolism of the rat, muscular activity must be ruled out. Our rats were studied in complete darkness and in light between 10 A.M. and 4 P.M., as we realized they would be reasonably quiet between these hours. In one series the oxygen consumption of four adult male rats was measured on 3 separate days, some days in the light and other days in the dark. In another series the carbon-dioxide elimination of young female rats was measured in consecutive half-hour periods with alternate exposure, 1 hour to the light and 1 hour to the dark.

The heat production of the male rats (table 3) was uniformly high on the first day, regardless of whether light or darkness prevailed. On the last 2 days, rat 5 had a heat production 10 per cent higher, rat 6, 4 per cent higher, and rat 8, 4 per cent lower in darkness than in light. Rat 7 showed no change. The kymograph records indicated that rat 6 was quieter in the light than in the dark, but on the whole it was not very restless in the dark. Rats 5, 7, and 8 were quiet both in the light and in darkness. The day to day

variations in heat production of these rats measured one day in the light and another day in the dark lie well within the limits of variation observed in measurements under standard conditions, and suggest that the basal metabolism of the rat is unaltered by exposure to complete darkness for 4 to 6 hours during midday.

TABLE 3

The influence of light and dark on the metabolism of male rats¹

RAT NO AND AGE	WEIGHT .	CONDITION OF CHAMBER	ACTIVITY	HEAT PRODUCED PER 24 HOURS	
				Per 200 gm.	Per square meter
	<i>Gm.</i>		<i>Per cent</i>	<i>Cal.</i>	<i>Cal</i>
No. 5					
262 days	503	Light	8	18.5	807
274 days	507	Dark	5	17.7	775
281 days	510	Light	4	16.0	701
No. 6					
262 days	439	Dark	10	16.6	690
274 days	434	Light	7	15.1	626
281 days	433	Dark	16	15.6	651
No. 7					
292 days	474	Light	5	16.6	714
299 days	473	Dark	6	15.5	661
306 days	473	Light	4	15.5	665
No. 8					
287 days	446	Dark	7	18.2 ²	763 ²
294 days	440	Light	7	14.8	620
301 days	431	Dark	7	14.4	595

¹ Measurements were made at 28°C., between 10.30 A.M. and 3.00 P.M., and 24 hours after food.

² During this experiment the rat broke a hinge on its cage door

In the second series (table 4) female rats were studied that weighed one-third as much as the males in the first series. The average heat production of rats 9 and 10 in experiment I was 30.4 calories per 200 gm. per 24 hours during the first hour with exposure to light. During the next hour, in the dark, it decreased to 26.8 calories and remained essentially at that level during the third hour with exposure to light.

In the second experiment, the average heat production of rats 11 and 12 was approximately 32 calories both during $\frac{1}{2}$ hour in the light and 1 hour in the dark. Throughout the next 3 hours, when the rats were in the light the first hour, in the dark the second hour, and in the light again the third hour, the metabolism was considerably lower, about 25

TABLE 4

The influence of light and dark on the metabolism of female rats¹ (18 to 24 hours after food)

EXPERIMENT NO., RATS, AND WEIGHTS	TIME OF DAY (30-MINUTE PERIODS)	CONDITION OF CHAMBER	AC- TIVITY ²	CO ₂ PRODUCED PER 30 MINUTES	HEAT PRODUCED PER 24 HOURS	
					Per 200 grams	Per square meter
			<i>Per cent</i>	<i>Gm.</i>	<i>Cal.</i>	<i>Cal.</i>
Experiment I Rat 9, 139 gm. Rat 10, 154 gm.	10.24 A.M.—10.54 A.M.	Light	6	0.2863	30.4	882
	10.54 A.M.—11 24 A.M.	Light	2	0 2723		
	11 37 A.M.—12.08 P.M.	Dark	0.2445	26.8	777
	12.08 P.M.—12.38 P.M.	Dark	0.2466		
	12 40 P.M.— 1.10 P.M.	Light	1	0.2360	26 0	754
	1 10 P.M.— 1.40 P.M.	Light	2	0.2415		
Experiment II Rat 11, 152 gm. Rat 12, 155 gm.	9 53 A.M.—10.23 A.M.	Light	13	0.3090	32 1	944
	10 27 A.M.—10.57 A.M.	Dark	...	0 3119	32.4	953
	10.57 A.M.—11 27 A.M.	Dark	0.3109		
	11.30 A.M.—12 00 M.	Light	0	0.2408	24.4	718
	12.01 P.M.—12.31 P.M.	Light	1	0 2283		
	12 34 P.M.— 1.04 P.M.	Dark	0.2525	25.4	747
	1.04 P.M.— 1.34 P.M.	Dark	0 2362		
	1.47 P.M.— 2.18 P.M.	Light	0.4	0.2428	25.0	735
	2.18 P.M.— 2.48 P.M.	Light	1	0.2380		
	2.51 P.M.— 3.21 P.M.	Dark	0.2771	27.4	806
	3.21 P.M.— 3 51 P.M.	Dark	0 2506		

¹ Two rats in same chamber, huddling, were measured at 30° to 31°C. in experiment I and at 29° to 30°C. in experiment II.

² Ascertained by ocular observation during the light periods only.

calories. During the last hour, in the dark, the metabolism was higher again. The heat production varied more with the time of day than with conditions of light and dark. Moreover the low metabolism observed during midday persisted throughout a short exposure (1 hour) to absolute darkness. Our findings for the rat are in accordance with the observations of Johnson ('26) who showed that the 24-hour rhythm of activity of wild deer-mice persisted in the absence of the daily change of light and dark.

INFLUENCE OF SEX AND AGE

The basal heat production at 30° of our adult female rats³ (nos. 1 and 2, 172 and 164 days old, table 2) was not far from 600 calories per square meter per 24 hours. Horst, Mendel, and Benedict ('30) have reported values of 600 to 700 calories for female rats about 200 days of age, measured at 28° and 24 hours after food. The heat production of male rats of the same age (unpublished data) has been found to be about 725 calories. The metabolism of our smaller females⁴ (evidently younger, but the ages are not known) averaged 765 calories⁵ (rats 9 and 10, table 4) and 733 calories⁶ (rats 11 and 12). According to Donaldson's ('24) standards these females, which weighed from 139 to 155 gm., were probably not far from 90 days old. The heat production of our male rats from 92 to 101 days old (table 7) averaged 786 calories per square meter. The average heat production of our four adult males at 280 days of age and weighing from 431 to 510 gm. (table 3) was 689 calories—a value similar to the 664 calories reported previously for the male rat weighing from 400 to 560 gm. (Benedict, Horst and Mendel, '32). Thus the metabolism of the young females was 5 per cent lower and that of the adult females, 17 per cent lower than that of the males, and with both sexes the metabolism decreased with

³From the Osborne and Mendel colony.

⁴From the colony of Prof. W. E. Castle at the Bussey Institution of Harvard University.

⁵On the basis of carbon dioxide produced from 11.37 A.M. to 1.40 P.M.

⁶On the basis of carbon dioxide produced from 11.30 A.M. to 2.48 P.M.

age. This same picture of decreasing metabolism with advancing age is shown in table 7.

THERMIC NEUTRALITY

In recent years many investigators have followed the excellent example of Hári ('24), Aszódi ('24) and others of the Tangl school at Budapest, who study the metabolism of the rat at an environmental temperature of 28°C. Examination of the literature, however, shows that the temperatures preferred in the different laboratories range from 26° (Asher and Honda, '27) to 32° and 33°C. (Terroine and Trautmann, '27). Fraser and Wiesner ('29-'30) have actually reported measurements on the rat at 37°C. Some of the variations in the conditions of thermic neutrality in the different laboratories can probably be explained by the different procedures employed in ascertaining the temperature of the environment. A thermometer near the rat will unquestionably be affected by the animal's temperature. It would be ideal to use a resistance thermometer wound entirely around the wire cage in which the animal is placed. The difficulty of incorporating this electrical equipment was so great that it was not used in our measurements. We experimented with a number of small thermometers attached to different parts of the animal's cage, one near the rat, one above it, one on the floor of the chamber (with the heating element functioning beneath the chamber), and one at the exit end of the chamber for the ventilating current and at least 2 cm. above the rat cage. The latter position was accepted as giving the most uniform temperature. The temperatures recorded by these four thermometers showed a difference of approximately 1°C. In all probability the records that we used of the temperature determined at least 2 cm. above the rat cage were within 1°C. of the average chamber temperature.

Goto ('23), Terroine and Trautmann ('27) and Houssay and Artundo ('29) have shown that above 28° or 30°C. the heat production of rats increases. Four of our male rats from 50 to 100 days of age were measured at 28° or 30° on

one day and at 32° on another day.⁷ Three males from 200 to 300 days old were likewise measured at 30° on one day and at 32°C. several weeks later. Some of the determinations were made with calcium chloride in the respiration chamber, to reduce the moisture content of the air. In another series

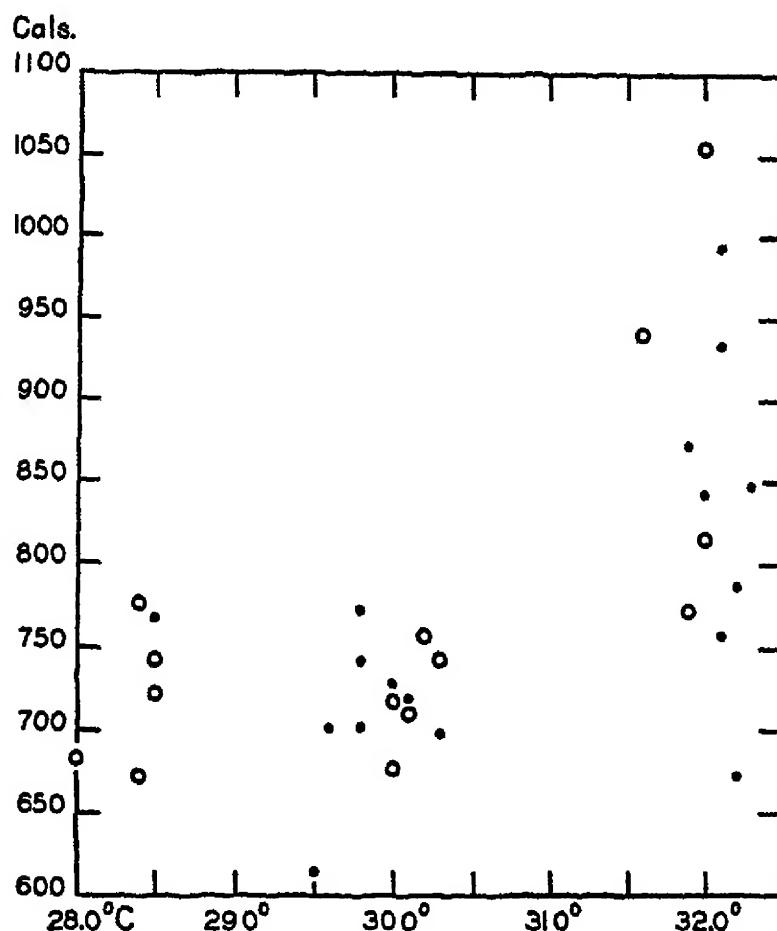


Fig. 1 Heat production of male rats per square meter of body surface per 24 hours referred to environmental temperature. The open circles represent determinations made without calcium chloride and the solid circles those made with calcium chloride in the respiration chamber.

the metabolism of three adult males about 240 days of age was measured at 30°, on some days with and on other days without calcium chloride in the chamber. The results are plotted in figure 1, the abscissae representing the environmental temperature and the ordinates the calories per square meter of body surface per 24 hours. The open circles represent the determinations made without calcium chloride and

⁷ The number of days between experiments varied from 4 to 21.

the solid dots those with calcium chloride. With one exception the metabolism of these rats was higher at 32° than at 28° or 30°C., the total increase averaging 30 per cent for three adult rats and two young rats or 15 per cent per degree (Centigrade) that the environmental temperature increased above 30°C. Three young rats measured at 28° and 32° had a total increase in metabolism of 10.5 per cent, or 2.6 per cent per degree increase in environmental temperature above 28°C. According to the kymograph records the young rats especially were more restless at 32° than at 28° or 30°C. Undoubtedly, the higher metabolism can be attributed to a great extent to the increase in activity. Thus it appears that with rats, measurements at 32° give too high results and that the most ideal temperature for measuring the basal metabolism of rats is at 28° to 30°C. Within these limits there is no appreciable variation in metabolism. With our young female rats (unpublished data) the metabolism was also essentially the same at 28° and at 30°C.

Humidity. It is conceivable that at 32° the moisture content of the air in the respiration chamber might have become excessive and that the normal path for heat loss by vaporization of water was disturbed. Greene and Luce ('31) did not observe a change in the percentage of heat lost by vaporization of water in the rat with variations in environmental temperature, but the range under observation, 25° to 31°C. was lower than the temperatures studied by us. Our observations were usually made with calcium chloride in the respiration chamber. When calcium chloride was not used, especially with a large rat, moisture frequently condensed on the inside of the glass chamber, even though the rate of ventilation was 2 liters per minute. A small tray filled with calcium chloride was placed upon the top of the cage above the rat, but at the opposite end from the thermometer so that the heat of absorption of water vapor could not influence the thermometer. As seen from figure 1, the metabolism at 32° was consistently high and that at 30° was always lower, regardless of the variations in the humidity as determined by the presence or

absence of calcium chloride. Our results confirm the earlier report by MacLeod ('07), who observed that at 30° the carbon-dioxide excretion in rats was the same with exposure to dry or moist air and at 33° it was higher than at 30°, both in dry and in moist air. Our experiments were far from a repetition of or an attempt to confirm the extensive studies of Sundstroem ('30), who compared the basal metabolism of rats living at 32° in an atmosphere having a relative humidity of 70 to 80 per cent with the metabolism of rats living at 32° in a dry atmosphere. Since Sundstroem used wet kata cooling power as an index of the climatic environment of his rats, it is impossible to compare his results with ours, as our method of temperature control was different.

The difficulty of obtaining measurements on the rat in complete repose, regardless of variations in temperature and humidity of the environment, complicates the study of the effects of environmental factors per se. In some of our experiments we attempted to obtain greater repose by caging the rats in perforated celluloid cylinders. When the rat fitted tightly into the cylinder, the metabolism was higher than normal. If the cylinder was slightly larger than the rat, the metabolism was essentially the same (unpublished data) as when the rat was measured in the regular metal cage. Frequently, the rat struggled in an attempt to change its position in the cylinder, at times resting on its back, at times on its side, but rarely ever in an upright position. Herzog ('30) with birds and Sundstroem ('30) with rats likewise found increased irritability and tonus when their animals were measured in cages small enough to prevent normal movements.

Rectal temperatures. The rectal temperatures of the adult rats determined immediately after the metabolism measurements were from 0.5° to 1.2°C. higher at an environmental temperature of 32° than at 30°C. The higher cell temperature of the rat would in itself tend toward an increased metabolism, in conformity with our findings with the respiration chamber.

THE INFLUENCE OF ENVIRONMENTAL TEMPERATURE UPON THE METABOLISM OF ADULT RATS

The basal metabolism of twelve male rats (127 to 150 days old) was established at 30°, when they had been living for 40 days at 21° to 27°C. The animals were then exposed to 16° for 10 days, when the metabolism was measured again at temperatures between 15.1° and 19.8°C. This procedure is in striking contrast to the quick transition from one temperature to another or the short exposure to the special environmental temperature, methods used, among others, by Benedict and Petřik ('30), Houssay and Artundo ('29) and Benedict and MacLeod ('29). Our rats had been used previously for a study of the effect of the protein level upon the basal metabolism. Our results introduce changes in metabolism, therefore, incident to the protein factor. The rats fasted, however, 24 hours before the metabolism was measured, so that the effect of protein was not an immediate after-effect corresponding to the specific dynamic action but a more persistent effect caused by changes in body composition resulting from extreme variations in the protein intake. The time between the measurements at the high and the low temperatures was so short that the age element did not enter to the extent that it did in the case of the growing rats discussed on pages 295 to 300. In estimating the increase in metabolism per degree fall in environmental temperature, we have used the exact chamber temperatures in each case (table 5) instead of the average values 30° and 17°C.

The data summarized in the first half of table 5 contribute information regarding the basal metabolism of the adult male rat, for the conditions of measurement, particularly as regards environmental temperature, were those stipulated for basal measurements. At 30°C. the heat production per square meter of body surface ranged from 603 to 742 calories. The latter value admittedly reflects the influence of activity greater than that noted in most of the other cases. The average metabolism of the entire series at 30° was 682 calories. Some values reported recently by other investigators are 718

TABLE 5
Metabolism of adult male rats¹ at 30° and 17° C.

RAT NO.	PROTEIN IN PREVIOUS DIET	30° C.				17° C.				INCREASE IN METABOLISM PER DEGREE FALL IN ENVIRONMENTAL TEMPERATURE	
		Weight	Rectal temperature	Chamber temperature	Calories per square meter per 24 hours	Weight	Rectal temperature	Chamber temperature	Calories per square meter per 24 hours	Calories	Per cent
		Gm.	°C.	°C.		Gm.	°C.	°C.			
28	Medium	230	37.4	29.7	672 ²	237	37.8	19.8	1243	58	8.6
23	High	274	37.3	28.9	643	297	37.9	19.4	1236	62	9.6
31	Low	250	37.1	29.6	631	240	37.1	19.0	1184	52	8.2
24	High	251	36.9	29.4	742 ²	266	37.0	17.6	1244	43	5.8
29	Medium	317	36.9	29.7	704 ²	300	38.2	17.2	1420 ²	57	8.1
26	High	317	36.9	29.1	686	295	37.6	15.9	1349	50	7.3
33	Low	256	37.6	29.8	603	238	38.3	15.9	1295	50	8.3
32	Low	197	38.0	30.0	651	213	38.8	15.6	1359 ²	49	7.5
30	Medium	282	37.3	29.7	707	266	37.3	15.4	1238	37	5.2
34	Low	254	37.0	29.5	693	249	37.0	15.4	1381	49	7.1
27	Medium	238	37.4	30.0	724	250	38.0	15.3	1350	43	5.9
25	High	284	37.2	30.0	725 ²	279	36.9	15.1	1328	40	5.5
Aver.		263	37.3	29.6	682	261	37.7	16.8	1302	49	7.3

¹ Rats measured when they had been 24 hours without food, at 28° or 16° C. (p. 278).

² Rat active more than 15 per cent of the time of measurement.

calories (Benedict and MacLeod, '29) and 805 calories (Verzár, '29).

The data in the second half of table 5 indicate the metabolism of the same rats at a relatively low environmental temperature of 17°C. and thus add to our general knowledge of the metabolism of animals of various sizes and zoölogical groups at 16° or 17°, the temperature at which Rubner made his measurements on different animals for formulating the surface-area law. Secondly, since the metabolism of these rats was likewise well established at 30°, the measurements at 17° give direct information with regard to the temperature coefficient or the increase in metabolism per degree fall in environmental temperature. The metabolism of our twelve rats was, on the average, nearly twice as great at 17° as at 30°C., 1302 calories as compared with 682 calories per square meter. It may be questioned whether values obtained at environmental temperatures ranging from 15.1° to 19.8° should be averaged. The heat values at the temperatures around 15° are higher, however, than those at the temperatures of 19°. Hence, the average value of 1302 calories probably represents what would have been the average metabolism of these rats had they been measured at 16.8°C. In only one instance did the activity exceed 20 per cent of the total time of measurement. Evidently there was no pronounced variation in activity ascribable to differences in environmental temperature.

The interval between the two temperatures at which the rats were studied varied from 9.5° with rat 23 to nearly 15° with rat 25. On the average the metabolism increased 7.3 per cent per degree decrease in environmental temperature below 30°C. This percentage increase corresponds to that noted by Houssay and Artundo ('29), who reported for 180- and 219-gm. rats a heat production of 86 calories per square meter per hour at 15° and 42 calories at 30°C., or an increase of 7 per cent per degree decrease from 30° to 15°C. Our rats were acclimatized to 16° and those of Houssay and Artundo were suddenly exposed to the low environmental temperature.

If the data reported by Benedict and MacLeod ('29) for rats at 10.9° to 31.7° are calculated on the basis of the measured minimum metabolism, the increase in metabolism is nearly 6 per cent per degree decrease in temperature below 28°C. Our rats acclimatized to 16° showed, on the whole, a greater increase in metabolism with exposure to low environmental temperature than that observed by other investigators who studied the immediate reaction of the rat to cold. The metabolic reaction of the rat to low temperatures resembles that of the dog and the guinea pig—species in which Rubner long ago found variations in metabolism with changes in environmental temperature.

The average value of 1302 calories per square meter of body surface noted with our twelve rats at 17° (based upon careful measurements with the most perfected technic) is presumably comparable to values found by Rubner and other research workers at 16°, the temperature at which the surface-area law was supposed especially to apply. Since our measurements were made during the period of minimum activity of the rat and in relatively short periods, it can be readily argued that 1302 calories represents the minimum value for the 24-hour metabolism at 17° and that if the rats had been measured during ordinary cage activity and over a 24-hour period, this value would have been very appreciably increased and would bear no relationship to the supposedly constant value of 1000 calories per square meter of body surface suggested for all warm-blooded animals at 16°C.

Rectal temperatures. The rectal temperatures of the twelve rats (table 5) at the end of 30 hours at 30° averaged 37.3° and ranged from 36.9° to 38.0°C. At 17° they averaged 37.7° and ranged from 36.9° to 38.8°C. Thus the rectal temperatures were lowest in the warm environment, but the differences were slight. The decrease in rectal temperature coincident with sudden exposure to 32° and 33° noted by Giaja, Chahovitch and Males ('28) represents the immediate reaction of the rat to a change in environmental temperature, whereas our experiments were concerned with the rectal tem-

peratures of rats acclimatized to two different environmental temperatures.

THE INFLUENCE OF ENVIRONMENTAL TEMPERATURE UPON THE
METABOLISM OF THE GROWING RAT

In a consideration of the effect of environmental temperature upon the metabolism of the growing rat at least two other factors must be recognized, constantly changing age and variation in size. It is usually assumed that differences in heat production caused by variations in size are equalized by expressing the results on the basis of body surface rather than body weight. However, variations in metabolism may occur with changes in age even at thermic neutrality. Moreover, the young rat may not react to low environmental temperature as does the adult rat. Hill and Hill ('13) found that at 14° the heat production per unit of surface area was 40 per cent higher in rats weighing 50 to 90 gm. than in rats weighing 90 to 130 gm. On the other hand, Mitchell and Carman ('26) did not find any essential difference at 28° to 31°C. in male rats from 91 to 188 days old. Houssay and Artundo ('29) observed that at 20° and at 0°C. small rats (100 to 179 gm.) had a somewhat higher metabolism per unit of surface area than large rats (180 to 320 gm.), but that at 30° the metabolism of rats weighing 100 to 320 gm. was essentially the same. Benedict and MacLeod ('29) noted that at 26° and 29°C. the metabolism of female rats from 60 days to over 2 years old was higher, the older the rat. The results obtained by Kayser ('30) in his excellent studies on rats (results recomputed by us from calories per kilogram to calories per square meter) show that at 29° three young rats and one adult had essentially the same metabolism, that at 21° a young rat had a lower heat production than an adult rat, and that the metabolism of his young rats was increased 7 per cent and that of his adult rats 11 per cent per degree decrease in environmental temperature from 29° to 20°C. The differences observed by the various investigators in the reaction of growing rats to low environmental temperatures

were probably caused by the different lengths of time that the rats lived at the particular temperatures. Our rats lived at a low temperature for several weeks before the metabolism was measured.

Four rats that had lived 7 days at 25° and were 42 to 44 days old were measured at 30°C. They then lived for 56 days at 20°, and their metabolism was measured at this temperature on the twenty-third and the fifty-sixth days when they were 66 and 99 days old, respectively. After 56 days at 20° they lived for 33 days at 25°, when they were again measured (average age, 133 days) at 30°C.

The first series of experiments made at 30° (table 6) show that with three of the 43-day-old male rats (the fourth rat was too active for comparative purposes) the average heat production per square meter of body surface was 913 calories. This is a relatively high metabolism in comparison with that of 850 calories observed on male rats 39 to 45 (table 7) of the same age and sex. Since the relatively high metabolism of 913 calories was noted with rats 35, 36, and 37 before they were exposed to 20°, the high heat production cannot be attributed to an after-effect of the stay at 20°C. The rats in both groups lived at 25°, were measured at 30°, and received the same diet, but rats 39 to 45 weighed considerably more (average weight, 90 gm.) at 45 days of age than rats 35 to 38 at the same age. In this and other papers the relatively great differences in weights of rats for the same age ascribable either to differences in feeding or to racial characteristics have presented difficulties in the method of calculating the basal metabolism for purposes of comparison, and challenge the legendary sufficiency of the calculations upon the basis of the surface area.

At 133 days of age (table 6) the rats measured at 30° had an average heat production of 725 calories and an average weight of 256 gm. Another group of rats, which may be considered as a control group (table 7), 135 days old and weighing 239 gm., had an average heat production of 756 calories or essentially the same as rats 35 to 38. Indeed, both these

groups of male rats had a metabolism not appreciably higher than the average of 708 calories previously reported (Benedict, Horst and Mendel, '32) for the adult male rat weighing from 300 to 400 gm. Therefore, only with the very young rats did the striking differences in weight and in metabolism

TABLE 6

The effect of environmental temperature upon the metabolism of growing male rats (24 hours after food)

RAT NO	AT 30° C. ¹				AT 20° C. ²			
	Age	Weight	Rectal temperature ³	Calories per square meter per 24 hours	Age	Weight	Rectal temperature ⁴	Calories per square meter per 24 hours
	Days	Gm.	°C.		Days	Gm.	°C.	
35	44	79	37.9	899	67	124	38.0	1243
36	42	71	37.9	891	65	111	38.1	1224
37	42	70	38.0	948	65	119	38.3	1018
38	44	68	38.2	*	67	107	37.8	1132
Aver.	43	72	38.0	913	66	115	38.1	1154
35	133	281	37.9	774	100	204	37.5	1105
36	133	260	37.9	739	98	171	38.0	1039
37	131	266	38.2	694	98	190	38.1	1040
38	135	216	38.2	692	100	173	38.1	1120
Aver.	133	256	38.1	725	99	185	37.9	1076

¹ Rats lived at 25° prior to metabolism measurements.

² Rats lived at about 20°C. for 56 days. For 23 days before first metabolism measurement the environmental temperature ranged from 18° to 22°C. and sometimes fell as low as 15°C. For 33 days before second metabolism measurement the environmental temperature was about 20°C., except for 10 days, when it rose to 25° at times and for 1 week when it was frequently 27°C.

³ At 43 days the rectal temperatures were determined when the rats were living at 25°C.; at 133 days after a 6-hour exposure to 30°C.

⁴ Determined when the rats were living at 20°C.

⁵ Rat active more than 15 per cent of the time of measurement.

for age appear. Because of these differences with the two groups of young rats, we find a difference in the age effect in the two series. Thus, according to the data in table 6, the average metabolism of the 43-day-old rats weighing 72 gm. was 26 per cent higher than that of the 133-day-old rats weighing 256 gm., whereas according to the data in table 7 the

TABLE 7
The metabolism of male rats, 42 to 138 days old, measured at 30°C., and 24 hours after food

RAT NO.	AGE	WEIGHT	HEAT PRODUCTION PER 24 HOURS	
			Per 200 gm.	Per square meter
	<i>Days</i>	<i>Gm.</i>	<i>Cal.</i>	<i>Cal.</i>
39	42	88	33.6	822
40	42	77	34.2	800
41	44	96	36.6	916
42	44	80	38.0	899
43	45	98	31.4	798
44	48	97	32.8	828
45	48	94	35.6	888
Average	45	90	34.6	850
46	57	114	34.0	907
40	57	102	32.5	834
47	59	118	30.1	808
41	60	138	32.0	909
48	62	100	33.4	852
49	64	125	30.5	855
48	64	134	25.8	727
50	69	125	29.2	803
Average	62	120	30.9	837
49	92	184	24.6	769
50	93	182	26.2	815
43	94	188	24.3	763
51	94	176	26.7	822
52	98	179	24.4	754
53	99	180	24.4	759
54	99	184	24.4	762
55	99	177	26.9	831
56	101	178	25.8	795
Average	97	181	25.3	786
57	131	236	21.4 ¹	724 ¹
43	133	236	21.0	713
58	133	237	20.2	688
51	133	248	23.6	816
40	134	224	25.5	851
56	136	247	21.7	749
47	136	252	22.3 ¹	774 ¹
59	137	249	21.0	725
60	138	226	22.8	763
Average	135	239	22.2	756

¹Rat active more than 15 per cent of the time of measurement.

average metabolism of 45-day-old rats averaging 90 gm. in weight was only 12 per cent higher than that of 135-day-old rats weighing 239 gm.

The heat production at 20° can also be considered from the standpoint of variation in metabolism with advance in age and increase in weight, i.e., the metabolism at 66 days and 20° can be compared with that at 99 days and 20°C. On the basis of square meter of body surface, the metabolism of rats 35 and 36 was from 12 to 18 per cent higher at 66 than at 99 days, whereas the heat production of rats 37 and 38 was essentially the same at both ages. At thermic neutrality the average metabolism of eight rats from 57 to 69 days of age (table 7) was 6 per cent higher than the heat production of nine rats from 92 to 101 days of age. Thus at the low environmental temperature rats 35 and 36 showed a greater variation in metabolism and rats 37 and 38 less, as the age advanced from 66 to 99 days, than the average change in heat production of rats of similar ages but measured at thermic neutrality.

As the age element enters into the comparison of the values at 30° and 20° in table 6, the picture of the temperature effect is obscure, even though the results have been calculated per unit of surface area. At 66 days of age and 20° the metabolism was notably higher than at 43 days of age and 30°, in spite of the difference in age. Similarly at 99 days and 20° the metabolism was much higher than at 133 days and 30°, although in this comparison the age effect is reversed, the rats being younger when measured at 20° than at 30°C. To eliminate variations in metabolism caused by differences in age, the heat production of rats 35 to 38 at 20° may be compared with the heat production of other rats (table 7) of the same age, weight, and sex, measured at 30°C. The average values are as follows:

62 days	120 gm.	30°	837 calories per square meter
66 days	115 gm.	20°	1154 calories per square meter
97 days	181 gm.	30°	786 calories per square meter
99 days	185 gm.	20°	1076 calories per square meter

According to these averages, the metabolism of the 66-day-old rats increased 3.8 per cent and that of the 99-day-old rats 3.7 per cent per degree decrease in environmental temperature from 30° to 20°C. In our study of the effect of temperature on adult rats (table 5) the increase in metabolism per degree fall in environmental temperature was, on the average, 7.3 per cent. Hence, low environmental temperatures seemed to affect the young rats less. However, the young rats were measured at 20° whereas the adult rats were measured at lower temperatures averaging 16.8°. In any event the evidence is clear that the metabolic reaction of the young, growing rat to a low environmental temperature is not greater than that of the adult rat.

Rectal temperatures. The rectal temperatures (table 6) of the rats at 20° were much the same as those at 25° or 30°, averaging essentially 38.0°C. at both environmental temperatures. However, the range (37.5° to 38.3°) in the measurements at 20° was somewhat greater than the range (37.9° to 38.2°) in determinations at 25° or 30°C.

SUMMARY

The oxygen consumption of two adult female and two young male rats in the first 20 hours after food (2 A.M. to 10 P.M.) was high at the start and reached a minimum slightly above the basal level at midday. After 4 P.M. the metabolism increased again and in the last hours of measurement was from 13 to 50 per cent higher than during the middle of the day. The rats were restless before 6 A.M., quiet during the middle of the day, and active after 4 P.M. The 24-hour heat production of the three rats per 200 gm. of body weight, calculated from the oxygen consumption measured between 2 A.M. and 10 P.M., was from 13 to 31 per cent above the basal heat production calculated from the oxygen consumption measured under conditions of repose between 10 A.M. and 4 P.M. Hence, for comparative purposes, the basal metabolism of the rat should be measured only between 10 A.M. and 4 P.M.

The metabolism of four adult male rats and four young females did not vary with changes from daylight to complete darkness between 10 A.M. and 4 P.M.

The metabolism of adult male rats increased, on the average, 7.3 per cent per degree decrease in environmental temperature below 30°C. That of young, growing male rats increased only 3.8 per cent.

The rectal temperatures of adult males averaged 37.7° at an environmental temperature of 17° and 37.3° at 30°C. The rectal temperatures of young, growing male rats were essentially the same (38.0°C.) at 20°, 25°, and 30°C.

At thermic neutrality the metabolism of female rats was lower than that of males, both at young and adult ages, and with both sexes the metabolism decreased with age.

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ANALYSIS OF THE COMPARATIVE FEEDING TRIAL BY THE VARIANCE AND COVARIANCE METHODS ¹

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ONE FIGURE

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Where but a single comparison has been involved in a feeding trial, i.e., where there were but two comparative lots, statistical analysis of such data as gains, feed consumption, or gain per unit of food eaten may be made either by a consideration of the differences between pairs of replicates (Student's method) or by the analysis of variance as proposed by Fisher. In fact, if the animals were allotted by pairs, the former method may prove quicker of calculation and hence be preferred in such cases.

But whenever three or more treatments have been involved in the study, i.e. three or more comparative lots, then, providing the requirements of random sampling were satisfactorily met in the allotment and that the necessary data for the individuals comprising each of the groups were recorded, analysis by the variance method will be found more efficient, in that it affords a larger number of degrees of freedom for estimating 'experimental error.' Fisher and Wishart ('30) state: "A valid test could be made based on only one degree of freedom for errors, but . . . the values of Z exceeded in 5 per cent or 1 per cent of trials are very large, and in conse-

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(J. A.)

quence relatively large real effects must be judged insignificant or, in other words, escape detection. Precision begins to be lost seriously when the number of degrees of freedom for error falls below 10."

The difference in degrees of freedom available for error between ordinary feeding trials of two, three, or four lots each having ten animals per lot may be illustrated as follows:

Variance due to	DEGREES OF FREEDOM		
	Two lots of ten animals	Three lots of ten animals	Four lots of ten animals
Between treatments (lots)	1	2	3
Between replicates	9	9	9
Error	9	18	27
Total	19	29	39

It will be seen from the above that each lot over two contributes nine additional degrees of freedom on which to estimate the error applicable to comparisons of the trial. In other words, the variability of the whole trial, less that part which can be accounted for as being due to specific and measurable factors, may be used as a basis for estimating the significance of differences between means of any two comparative lots.

The extent of this variability is best measured by the variance (s^2), for it is a characteristic of this measure that the final or total variance within a group is the sum of the variance contributed by the several independent factors causing variability.

The analysis of variance as applied to a comparative feeding trial then is simply the dividing up of the total variance of the trial into that due to each of the known causes and to that remaining or chargeable to the total of the unknown or unmeasured causes or 'experimental error.'

In every comparative feeding trial there is one cause of variability, the imposed experimental condition, and usually at least one other which is known and measurable. Thus in the statistical analysis of such trials we usually will divide the total variance into that: 1) between treatments, 2) between replicates and 3) remainder or 'error.'

Thus, if live weight gains between lots are under consideration, an analysis of the variance of these data will group under 'error' the results of all factors which have affected the live weight gains, other than those accounted for under such headings as 'between replicates.' Among such factors, the one of greatest importance in normal cases will be the amounts of feed eaten by different individual animals, and obviously the value of any study of the average gains of the groups differently treated will be enhanced if the effects of differences in quantity of feed eaten can be eliminated. The most usual method of eliminating the effect on gains of differences in feed consumption is to calculate the gain per unit of feed eaten, and to use this ratio as the basis of the analysis of the efficiency of the experimental rations in producing live weight increases.

Data from a comparative hog feeding trial recently completed at Macdonald College may be used to illustrate the methods of analysis herein discussed and table 1 gives feed consumption, live weight gains, and the calculated gain per 100 pounds of feed eaten for each pig in the aforementioned test. The totals for rows and columns are also included together with the actual and relative means for the columns.

Another method of freeing the live weight gains of the effects of varying feed consumption which may be used in trials which include data for individual food intake is by the statistical procedure known as the analysis of covariance. This method, by means of the regression of gain on feed eaten, corrects the variance of the gains to account for the effects of varying feed intake. In this way that portion of the variance in gains both between treatments and between replicates, due to difference in feed intake is eliminated. It has the advantage over the gain-feed ratio method not only of avoiding the calculation of the ratios of each animal and the errors in interpretation which may result from the use in analysis of such calculated ratios, but also of yielding at the same time separate information relative to the actual gains, the feed consumption, and the degree of correlation between them.

TABLE 1
Feed eaten, live weight gains, and gain per 100 lbs. feed eaten—data from hog feeding trial no. 33 B. Macdonald College

	LOT I			LOT II			LOT III			TOTALS OF ROWS (t_2)		
	Lbs feed eaten (X)	Lbs gain (Y)	Gain per 100 lbs feed (Z)	Lbs feed eaten (X)	Lbs gain (Y)	Gain per 100 lbs feed (Z)	Lbs feed eaten (X)	Lbs gain (Y)	Gain per 100 lbs feed (Z)	(X)	(Y)	(Z)
	382	66	17	377	88	23	441	90	20	1200	244	60
	335	72	22	403	92	23	399	85	21	1137	249	66
	388	84	22	376	86	23	362	75	21	1126	245	66
	316	47	15	381	90	24	372	82	22	1069	219	61
	319	75	24	345	85	25	357	85	24	1021	245	73
	399	87	22	359	82	23	367	79	22	1125	248	67
	358	75	21	360	80	22	396	93	23	1114	248	66
	355	73	21	352	96	27	349	72	21	1056	241	69
	344	59	17	331	84	25	380	85	22	1055	228	64
	339	70	21	327	77	24	346	81	23	1012	228	68
Totals of columns (t_1)	3535	708	202	3611	860	239	3769	827	219	10915	2395	660
Means	353.5	70.8	20.2	361.1	86.0	23.9	376.9	82.7	21.9	363.83	79.83	22.0
Means on relative basis	94.4	88.7	91.8	99.3	107.7	108.6	103.6	103.6	99.5	100.00	100.00	100.0

A discussion of the mathematical theory behind this method is not a part of this paper. For such the reader is referred to references cited at the end of this paper. As an example of its application to the ordinary comparative feeding trial, however, the data from the same experiment involved in the analysis of variance shown in table 2 have been analyzed by the covariance method and are presented in tables 3 and 4 following.

Table 2 gives the analysis of variance for the gain per 100 lbs. of feed eaten. Comparison of the difference between the $1/2$ log values for 'error' and 'treatment' (between lots) with the Z value necessary for odds of 20 to 1 shows that the differences between lots due to the experimental treatments are sufficiently greater than those expected from chance factors to justify us in claiming significantly different gains between lots per 100 pounds feed eaten.

In order to obtain a quantitative estimate of the differences between these three treatments we may, through the calculation of the standard deviation for error, determine the amount of difference which must exist between the means of any two of these lots to cover the allowance necessary for variation due to chance (or error). Such calculation indicates that some 6.6 per cent of difference is required to cover error (odds of 20 to 1) or, in other words, no differences due to treatment of less than 6.6 per cent are measurable in this trial.

In table 3 are given the analyses of variance of feed consumption (3 a), variance of gains in weight (3 b), and covariance of gains and feed consumption (3 c). The analyses in tables 3 a and 3 b in so far as the sums of squared deviations and variance (s^2) are concerned are carried out as in any ordinary analysis of variance. The calculation of the standard deviation (s) and the coefficients of variability (C.V.) are included as additional information concerning the feed consumption and gains of the animals during the trial. In table 3 c the sum of the products of gain and feed, $S(XY)$, is obtained by procedure similar to that involved in the calculation of sums of squares for either variable (X or Y) singly,

the difference being that instead of squaring the value in question (as pounds feed), the product of two corresponding values is found (as pounds feed \times pounds gain). The correction for the mean is made by subtracting from the raw sum

TABLE 3

Analysis of variance and covariance of gains and feed consumption

<i>Table 3 a. Feed eaten (X)</i>						
VARIANCE DUE TO	D/F	S(x ²)	VARIANCE (s ²)	s	GENERAL MEAN	C V
Between treatments	2	2853.50	1426.75			
Between replicates	9	10721.50	1191.28			
Error	18	9133.55	507.42	22.52	363.83	6.4%
Total	29	22708.55				
<i>Table 3 b. Gains in weight (Y)</i>						
VARIANCE DUE TO	D/F	S(y ²)	VARIANCE (s ²)	s	GENERAL MEAN	C V.
Between treatments	2	1279.27	639.63			
Between replicates	9	334.16	37.13			
Error	18	1540.72	85.60	9.25	79.83	11.46%
Total	29	3154.15				
<i>Table 3 c. Covariance of feed eaten and gains (XY)</i>						
COVARIANCE DUE TO	D/F	S(xy)	MEAN COVARIANCE	r _{xy}	b'	b''
Between treatments	2	1143.11	571.55	0.5983	(0.4006)	(0.8936)
Between replicates	9	980.16	108.91	0.5173	(0.0914)	(2.9332)
Error	18	2832.28	157.35	0.7550	0.3101	1.8382
Total	29	4955.55			0.2182	1.5711

Sums of squares of gains corrected for feed eaten = $S(y^2) - 2(b' \text{ for error})(Sxy) + (b' \text{ for error})^2 S(x^2)$.

Sums of squares of feed corrected for gains = $S(x^2) - 2(b'' \text{ for error})(Sxy) + (b'' \text{ for error})^2 S(y^2)$.

of products the quantity obtained by multiplying the total of the one variable (as feed) by the mean of the other (as gain).

The mean covariance is obtained by dividing the sum of the products $S(XY)$ by the degrees of freedom in each case. Column 5 in this table gives the correlations between gains

and feed eaten in this trial, all of which are significant. A significant value of r is of interest in showing that a portion of the variance in gains is associated with differences in feed consumption.

These values are obtained by the formula:

$$r_{xy} = \frac{\text{covariance}_{xy}}{\sqrt{s_x^2} \cdot \sqrt{s_y^2}}$$

From these data the two regression coefficients (b' , b'') were also calculated:

$$b' = \frac{\text{covariance}_{xy}}{s_x^2}$$

and

$$b'' = \frac{\text{covariance}_{xy}}{s_y^2}$$

The former gives the average number of units of change in the gains with every unit change in feed consumption, while the latter (b'') is the average change in feed consumption with each unit change in gains. It will be seen that each pound increase in feed eaten resulted in an increase of about one-third of a pound in gains. ($b' = 0.3103$).

These covariance and regression values may now be used to correct the analysis of the gains (or feed eaten) to account for differences in feed consumption (or gains). That is, by adjusting the variance of the gains for the effect of feed eaten, we obtain an analysis of gains with the effect of varying feed consumption eliminated as well as may be by a linear relationship. The formulae for calculating the corrected sums of squares are appended to table 3 and the corrected values are found in table 4. It should be noted that one more degree of freedom for error has been lost through the calculation of the constant (b) from the residuals.

Analysis of variance of gain-feed ratio vs. analysis of gains (or feed) by covariance

In the example used it will be seen that the two methods of eliminating from the live weight gains the effects of varying feed consumption, i.e. analysis of the calculated gains per 100

pounds feed eaten vs. analysis of gain and feed eaten by covariance, appear to have given approximately the same degree of precision. In table 2 it is shown that differences between gains

TABLE 4
*Analysis of variance of gain and feed consumption corrected by regression
(see table 3)*

VARIANCE DUE TO	DEGREES OF FREEDOM	FEED EATEN—(CORRECTED FOR GAINS)			GAINS—(CORRECTED FOR FEED CONSUMPTION)		
		S(x ²)	s ²	1/2 log _e s ²	S(y ²)	s ²	1/2 log _e s ²
Between treatments	2	2973.60	1486.80	3.6522	844.71	422.35	3.023
Between replicates	9	8247.16	916.35	3.4102	757.27	84.14	2.216
Error	17	3927.78	231.00	2.7212	662.45	38.97	1.820
Totals	28	15147.78			2264.42		

Necessary Z for P = 0.05 where n₁ = 2; n₂ = 17 is 0.6393

	FEED EATEN		GAINS	
	Pounds	Per cent	Pounds	Per cent
Standard deviation for error	15.20	4.18	6.24	7.81
Standard deviation for single difference	21.49	5.91	8.83	11.06
Standard error for difference between means of 10	6.80	1.87	2.79	3.49
Necessary difference between means of 10:				
t (for n = 17; P = 0.05) = 2.11	14.35	3.95	5.88	7.37
t (for n = 17; P = 0.10) = 1.74	11.83	3.25	4.86	6.09

Differences

	COMPARISONS	PER CENT DIFFERENCE ¹	NECESSARY DIFFERENCE	NET DIFFERENCE	FAVOR OF
Gains	Lot I vs. II	16.15	+ 6.09	10.07	Lot II
	Lot I vs. III	5.87	± 7.37	0	..
	Lot II vs. III	10.28	+ 6.09	4.19	Lot II
Feed	Lot I vs. II	5.59	+ 3.25	2.34	Lot I
	Lot I vs. III	0.42	± 3.95	0	.
	Lot II vs. III	6.01	+ 3.25	2.76	Lot III

¹ Differences based on corrected lot means.

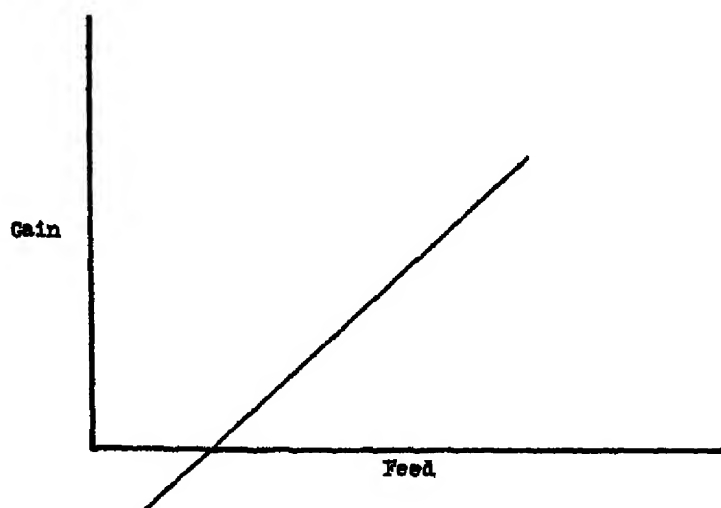
per 100 pounds feed eaten greater than 6.6 per cent can be measured and in table 4, where by covariance, effects of varying feed consumption have been corrected for, differences between gains greater than 6.09 per cent may be detected in this same trial.

TABLE 5
Summary of tables 2 and 4

METHOD	LOTS COMPARED	OBSERVED DIFFERENCE, PER CENT	NECESSARY DIFFERENCE, PER CENT	DIFFERENCE PROVED STATISTICALLY AS CREDITABLE TO EXPERIMENTAL TREATMENT
Gain per 100 lbs. feed eaten	I vs. II	16.8	6.6	10.2
	I vs. III	7.7	6.6	1.1
	II vs. III	9.1	6.6	2.5
Live weight gains corrected by covariance for varying feed consumption	I vs. II	16.2	6.1	10.1
	I vs. III	5.9	± 7.4	0.0
	II vs. III	10.3	6.1	4.2

This close agreement, however, is probably coincidental rather than to be expected, for the original calculation of the gain-feed ratio introduces a systematic error of variable magnitude which will be reflected in any analysis of the variance of this ratio. The reason for this error is that it is necessary to supply a pig with a certain amount of feed in order merely to keep it alive, without gaining in weight at all.

In other words, the line of regression of gain on feed consumed (assuming it to be linear) does not pass through the origin but looks something like this:



Consequently even if the correlation between gain and feed eaten were perfect so that the gain were uniquely defined as a linear function of the feed, that function would not be of the form $g = b f$, but rather $g = a + b f$. The gain per 100 pounds feed then becomes:

$$\frac{100 (a + b f)}{f}$$

which is not a constant but a variable quantity tending to the limit b as f is increased indefinitely. As in practice f cannot be increased indefinitely, the values of the ratio obtained will be subject to systematic errors.

The extent of this error will depend upon the magnitude of the variations in feed consumption between pigs, the greater the differences in the amounts of feed eaten the greater the error, and consequently the greater the discrepancy between the results of the two methods of analysis.

The objection above raised to the gain-feed ratio as usually calculated applies particularly to its use as a basis for statistical analyses.

As a method of describing the end result of a feeding test in practical terms, the average gain made on a unit of feed (or the average feed required to produce a unit of gain) is often quite useful. When this calculation is made for each pig of the trial, however, a statistical analysis of the array of these ratios will include, in addition to the experimental error, a systematic error which may be of sufficient magnitude to seriously distort the result, and vitiate the estimate of experimental error and hence any test of significance.

The covariance method, in which the variance of the actual gains, freed from the effects of varying feed consumption by regression, is subjected to analysis, is sound both biologically and statistically. It gives the best estimate possible from the data of the significance of such differences as may exist between comparative lots. Either the gains, with the effects of varying feed consumption eliminated, or the feed eaten, with the effects of different gains made eliminated, or both, may be studied by this method. In the former case, the re-

gression of gain on feed would be used in the regression equation (b' in table 3 c). In the latter, the regression of feed on gain would be used (b'' in table 3 c).

It should also be noted that where the estimate of error is obtained by an analysis of covariance, the differences between lot means must be based on corrected lot means. These corrected values may be determined by correcting the deviations of the lot means from the general mean for differences in feed consumption. The corrected deviations will be:

$$\bar{Y}_1 - \bar{Y} - b (\bar{X}_1 - \bar{X})$$

in which $\bar{Y}_1, \bar{Y}_2, \dots, \bar{Y}_n$ are the mean gains of lots 1, 2, etc.; $\bar{X}_1, \bar{X}_2, \dots, \bar{X}_n$ the mean food consumptions of lots 1, 2, etc.; \bar{Y} and \bar{X} the general means for gain and feed eaten respectively; and b the regression for error of X on Y or Y on X as the case may be.

It would seem, therefore, that the covariance method leads to a more desirable interpretation of the gain and feed consumption data of a feeding trial than is obtained through the analysis of gain-feed ratios calculated for the individual experimental subjects. Its use, of course, is limited to trials in which individual feed consumption is recorded.

Application to other feeding trial data. While the example here presented of the use of covariance deals with feed consumption and live weight gains, the method is equally applicable to other correlated variables. Thus in the case of trials involving successive feeding periods where the treatment during an early period may have disturbed the gains and hence the initial weights of subsequent periods, the effects of differences in initial weight, which could not be corrected for by allotment, may be eliminated from the analysis by covariance.

The necessary difference. It may be in order here to call attention to the 't' value and 'Z test' used in estimating the allowance necessary between two means for experimental error. These values change according to the number of degrees of freedom available for the estimation of the standard

errors and also with the reliability demanded. Odds of 20 to 1 or a probability of 5 per cent have recently come to be accepted as sufficient reliability for ordinary comparative feeding trials.

In testing the significance of a mean difference we are testing whether or not the observed difference deviates significantly from zero. In an analysis of variance such a test is made by comparing the Z value necessary for the significance wanted, with the Z value obtained (i.e., the difference between the $1/2 \log$ values of the variances for 'between treatment' and 'error'). Differences between the $1/2 \log$ s greater than the necessary Z value indicate that treatments have caused differences between lots greater than those chargeable to error.

The same information may be obtained from the standard deviation for error in the trial, using instead of the Z values, a table of 't.' These values are simply the ratios which must exist between means and their standard errors for a given level of significance.

This latter is the test which must be used in comparing two means within a trial in which more than two lots are involved, for the Z test simply tells whether or not the variation between differently treated lots is significantly greater than that between animals treated alike (error). If the Z test is negative, then no further test is necessary; but if there is a significant effect of treatment indicated by this test, then the standard deviation for error is calculated (square root of the variance for error) and from it the standard error of a mean difference $\frac{s_x \sqrt{2}}{\sqrt{n}}$. In this case n is the number on which the means compared are based.

To get the necessary difference between two means the standard error of the mean difference (s_{md}) is multiplied by the appropriate 't' value, i.e. with n corresponding to the degrees of freedom from which the error of the trial is estimated and P equal to whatever odds are wanted.

$P = 0.05$ vs. $P = 0.10$ for odds of 20 to 1

The P necessary for odds of 20 to 1 will normally be at the 5 per cent point or $P = 0.05$. In cases, however, where by either the Z or t tests there has already been proved a positive or negative significant difference from zero due to treatment, the calculation of the necessary allowance for error (or necessary difference) with odds of 20 to 1 may be made using the t value given for $P = 0.10$. This is permissible since once it has been established that a significant difference of known sign exists, we need measure an allowance for error in one direction only, in which case the odds for a given P value as given in the table of t are doubled.

If 'net difference' is defined as the observed difference minus the allowance for experimental error, or the maximum difference which can be proved statistically to be due to the experimental treatment, then it follows from the above that the allowance for error used in determining the net difference will be based on the doubled P value, for unless a significant difference is established, there can be no 'net difference.' For example, the t value for $n = 17$ and $P = 0.05$ is 2.11, which in terms of difference indicates that two means of 10 in this trial must differ in gains by at least 7.37 per cent ($s_{\text{md}} \times t = 3.49 \times 2.11$) before such a difference can be considered to be due, in 95 per cent of the cases, to the experimental treatments and not to uncontrolled factors.

In comparing lots I and II, there is a difference of 16.8 per cent (based on corrected means) and if we use lot I as the base or check lot the difference is +16.8 per cent in favor of lot II. By our test we should consider that any difference greater than +7.37 per cent was significant. Our difference, therefore, is not only significant but positive. Had lot II been chosen as check, then the difference would have been negative.

Since we are now concerned with measuring how much the mean of lot II must be greater than that in lot I in 95 per cent of cases before we can begin to count the effects as due to feed or other experimental treatment, we may calculate

the 'net difference' by using a t value of 1.74, corresponding to $n = 17$ and $P = 0.10$.

But let us suppose that the difference between these lots had been 7.0 per cent. By our t test ($n = 17 : P = 0.05$) we should have no assurance that the true difference was 7 per cent in favor of lot II. It might just as truly have been 7 per cent in favor of lot I because by our test we have found that any difference, positive or negative, as great as 7.37 per cent might have occurred once in twenty times by chance alone. Therefore, in spite of the fact that by using the t for $P = 0.1$, we should obtain in favor of lot II a 'net difference' of $+ 0.91$ per cent ($+ 7.00 - 6.09$), the figure is based on the false premise that the observed difference was in favor of lot II, and that its magnitude was 7 per cent.

This distinction between the test of significance and the calculation of a 'net difference' after the significance of the observed difference has been demonstrated, becomes of particular importance in those border-line cases where it is sometimes possible to obtain an apparent 'net difference' when actually no significant effect due to the imposed experimental conditions can be claimed. On the other hand, to forego the use of the doubled value of P when it can legitimately be applied is to be conservative to the point of materially underestimating real differences creditable to the experimental treatments under consideration.

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THE BIOLOGICAL ACTIVITY OF SOME CAROTENE PREPARATIONS ¹

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ONE FIGURE

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The possibility of feeding carotene which had been adsorbed from solution by ultra-porous powders seemed attractive. By this method expensive isolation of carotene might become unnecessary for nutrition work.

The success of such feeding must depend upon the ability of the intestinal juices to desorb or remove the carotene from the adsorbing surface of the powders. Although Marcus ('31) observed that contact with fine powders destroyed vitamin A and Lachat, Dutcher and Honeywell ('30) stated that vitamin A in cod liver oil may be adsorbed on highly activated silica gel so tenaciously that it cannot be rendered available when the silica gel is fed to rats, more encouraging results were reported by Holmes, Lava, Delfs, and Cassidy ('33). These authors were able to recover a high percentage of vitamin A adsorbed on certain porous solids by the use of proper solvents. They obtained similar results with carotene but these experiments did not prove that animals could show an effective recovery of adsorbed carotene. Hence the feeding

¹ The nutrition studies were made possible by a generous 'Grant-in-aid' from the National Research Council.

experiments described below. As porous adsorbents it was decided to use ashless norit (Miller, '26), activated alumina prepared by the Aluminum Company of America, and air-floated silica gel obtained from the Silica Gel Corporation.

Carotene and carotene-containing materials prepared in the Oberlin laboratory were sealed in glass tubes under nitrogen or carbon dioxide and received in the Illinois laboratory during the years of 1931 and 1932. The materials were fed to vitamin-A-depleted rats in order to determine to what extent the carotene was utilizable by the animal body as the sole source of vitamin A in the diet.

The preparations were stored at 1° to 10°C. from the time they were received. After the seals had once been broken, two-hole rubber stoppers fitted with stop-cock tubes were inserted into the mouths of the test tubes to facilitate maintenance of a nitrogen atmosphere during the time material was being removed for daily weighings and preparatory to continued storage under nitrogen. Commercial nitrogen was bubbled through alkaline pyrogallol to remove traces of oxygen, then through distilled water, and finally through soda lime.

EXPERIMENTAL

The curative method was employed, using young albino rats 3 to 4 weeks old of such dietary history that the first symptoms of xerophthalmia were exhibited after 21 to 28 days on the vitamin-A free regimen (Meyer and Hetler, '29). The appearance of a small line extending from the medial corner of the eye along the upper lid was found to be a reliable sign of vitamin A depletion (in no instance were the animals allowed to develop severe xerophthalmia). This sign marked the beginning of the 8 weeks' experimental feeding period. In all cases weighed amounts of the test materials were fed daily. In the later experiments, as will be indicated, the test materials immediately after weighing were placed directly into the mouth of the rats in order to minimize the possibility of the destruction of activity by exposure to air.

Careful observations were made of food intake, of weight increment, and of symptoms typical of vitamin A deficiency. Autopsies were made on all animals with special examination of the kidneys and urinary bladder for stones and of the glands at the base of the tongue for evidence of purulent material.

Several different types of control animals were used. They were as follows: negative controls, i.e., animals receiving the basal diet and no source of vitamin A; positive controls, i.e., animals reared on the same diet and cured of vitamin A deficiency symptoms by the addition of cod liver oil or pure carotene dissolved in highly purified ethyl laurate;² and control animals fed the ethyl laurate without the carotene.

DISCUSSION

1. In a purely preliminary series of tests the following preparations were fed: A, C, and D, with carotene adsorbed on silica, sample B with carotene adsorbed on carbon, and sample E, with carotene adsorbed on alumina. When these materials were fed to the vitamin A depleted rats for a period of 17 to 20 days in daily amounts representing a large excess of carotene, the animals were in no better nutritive condition than were the negative control rats. The xerophthalmia was not alleviated but became increasingly severe; hematuria persisted in a large number of the animals; infections of neck glands resulting in pus sacs were frequently encountered; and failure of appetite and loss of body weight always occurred.

The supplements were well eaten at first and in some cases throughout the entire test period, but in most cases they were later entirely refused. (Other control animals with severe xerophthalmia were cured within the same period with cod liver oil or crystalline carotene dissolved in ethyl laurate.) At the end of 17 to 20 days the supply of test material was exhausted partly due to the attempts to increase the dosage sufficiently to effect a cure. Cod liver oil was then given to

² Ethyl laurate no. 904, Research Lab. Eastman Kodak Co., Rochester, New York

these severely deficient animals, and in every case a rapid increase in body weight resulted, although the xerophthalmia was not cured. In the thirty-four animals used kidney stones were found in two and pus sacs at the throat in three cases. The eight negative control animals from the same litters lived, as an average, 47 days after the experimental period began. Kidney and bladder stones were found in every case and pus in the glands at the base of the tongue in five of the animals. Three animals receiving cod liver oil, and seven receiving crystalline carotene in ethyl laurate after the first appearance of xerophthalmia, were of normal weight, sleek, and free from any pathological lesions at the end of the 8 weeks' test period. (Carotene was fed at levels of 0.05 mg. and 0.1 mg. daily to these animals.) No evidence could be found that any one of the samples A, B, C, D, or E supplied utilizable carotene as a source of vitamin A to the animals.

These purely preliminary experiments showed us that 0.33 mg. of carotene adsorbed (from petroleum ether) on each gram of silica gel and even as much as 0.83 mg. per gram of alumina, or 1 mg. per gram of norit carbon were not made available by the digestive juices of rats. Consequently, it was decided to increase the concentration of carotene to 3 mg. per gram of alumina. This adsorbent was selected for the final series because of its laboratory suitability for adsorption and recovery.

2. Samples H, F, and M were so prepared that 3 mg. of carotene were adsorbed on each gram of alumina. In the case of F the carotene adsorbed from petroleum ether in the presence of ethyl laurate, and of M in the presence of cholesterol.

The solvent was not quite completely removed by distillation at 50° with reduced pressure in an atmosphere of CO₂. Preliminary to adsorption the alumina, ground to 200 mesh, was activated for 3 hours in a stream of CO₂ to remove air, moisture, etc. Activation in the preliminary series was far less thorough and there may have been some loss of carotene by oxidation. Two levels of feeding were chosen in each case;

i.e., 0.08 gm. of the activated alumina carrying 0.24 mg. carotene, and 0.15 gm. of the activated alumina carrying 0.45 mg. of carotene. Immediately after the day's dose was weighed it was hand fed by means of a small spatula into the mouth of the animal. All three samples were found active at both levels fed. Figure 1 illustrates the resulting average weight increments which occurred in all cases. The negative control litter mates (twenty-one rats) were all dead before, or a few days after, the end of the 8 weeks' test period. Six animals in this series received 0.005 mg. daily of carotene dissolved in ethyl laurate. These animals did not gain as much weight as did the six animals receiving one drop of cod liver oil daily, nor was xerophthalmia as rapidly or completely cured in the carotene fed group. The growth of rats receiving the crystalline carotene in ethyl laurate was no better and in some cases not as good as that of the animals receiving test materials H, F, and M when the latter samples were used at the higher level of feeding. The nutritive condition of the animals receiving 0.15 gm. daily of test material, H, F, and M was decidedly better than that of the rats receiving the smaller amount, i.e., 0.08 gm. daily. This improvement was especially noted in the cases of test materials M and F. The animals receiving the 0.08 gm. daily of test material F were in poorest condition of all the animals (except the negative controls) but where the larger amount of test material F was fed the animals were in a better condition than were those in any of the other groups.

Activity of recovered carotene

At the two levels fed the animals were receiving approximately forty-eight and ninety times as much carotene as the animals receiving the 0.005 mg. daily dosage. It appeared that the carotene had either lost some activity during the adsorption process, or that it was only partially accessible to the animal from the adsorbents used. In order to determine whether the physiological activity of the carotene had been affected by the adsorption process, carotene which had been

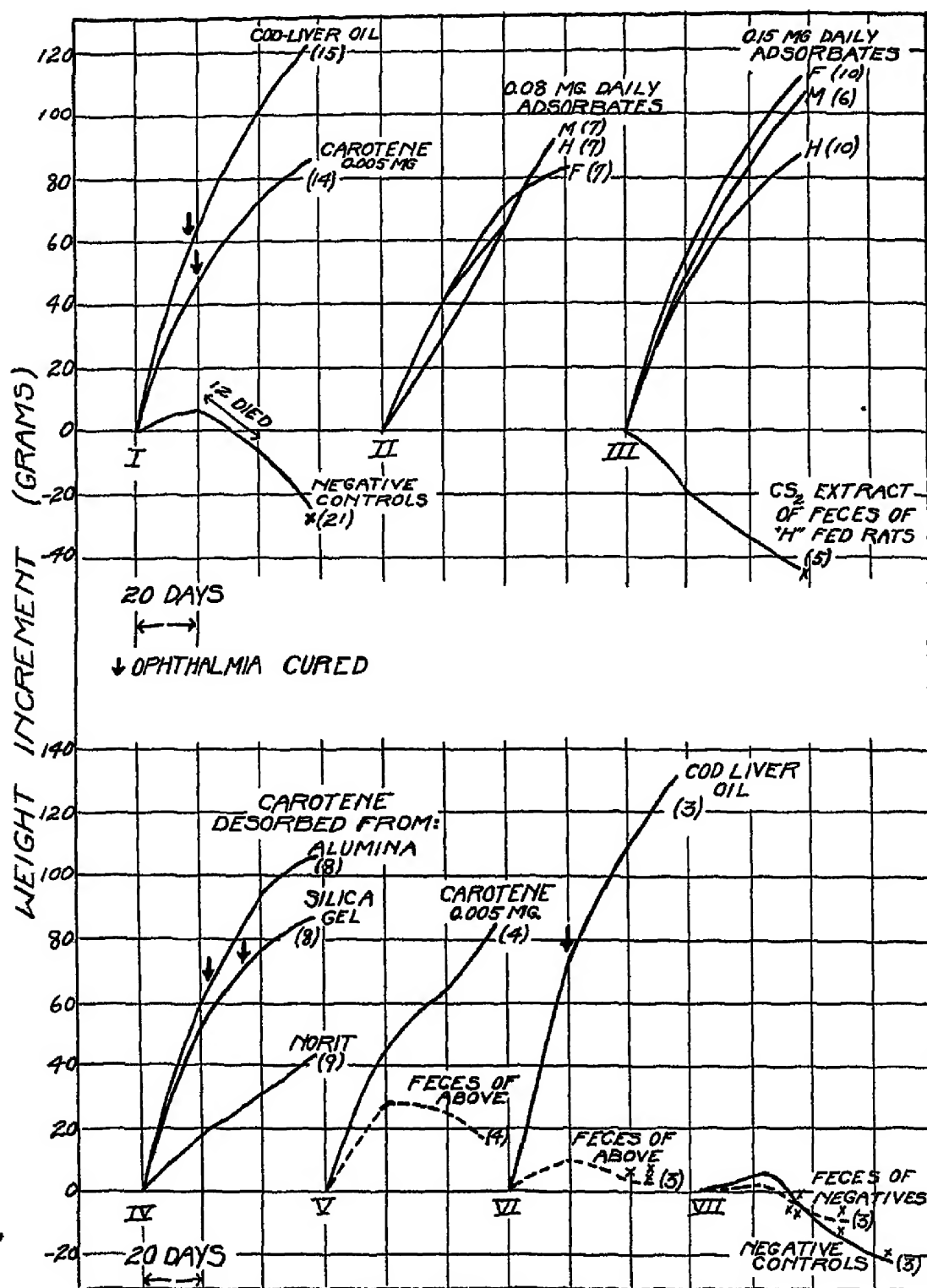


Fig. 1 Composite weight increment curves of rats receiving vitamin A free diets alone or supplemented with cod liver oil, with carotene, or with carotene preparations. Growth is plotted from the time definite xerophthalmia developed at which time the experimental test diet was begun. The number of animals represented is indicated in parentheses at the end of each curve.

I. The fifteen animals fed one drop (18 mg.) of cod liver oil daily grew at a normal rate and moderately severe xerophthalmia was entirely cured in from

desorbed or recovered from activated silica gel, alumina, and carbon was fed to other vitamin A depleted rats. The material was prepared in the Oberlin laboratory by methods previously described by Holmes, Lava, Delfs and Cassidy ('33). Carotene was adsorbed from petroleum ether and desorbed by chloroform except in the case of norit where carbon disulfide was used for recovery. Evaporation of the solvent at 50° under reduced pressure left a slight amount of solvent in the residue. In each case the recovered pigment (determined colorimetrically) was fed in ethyl laurate solution. The animals were given 0.006 mg. of recovered carotene daily and the results in average weight increment with each test material are graphically presented in figure 1, group IV. The

2 to 20 days The fourteen animals receiving 0.005 mg. daily of carotene dissolved in pure ethyl laurate grew at a slower rate than the cod liver oil-fed rats; xerophthalmia was cured in all but three cases in from 3 to 20 days. Xerophthalmia became increasingly severe in every case where no source of vitamin A was given (negative controls). Twelve animals died 20 or 40 days after the depletion symptoms appeared and all but one were dead before the end of the 8 weeks' test period.

II and III When carotene adsorbed on alumina alone 'H,' or on alumina in the presence of ethyl laurate 'F,' or on alumina in the presence of cholesterol 'M,' was fed at the lower level (0.08 gm. daily of material representing 0.24 mg. of carotene) growth was comparable to that of the animals receiving 0.005 mg. of carotene daily. The xerophthalmia was not as promptly cured, however, in the cases where the carotene on the adsorbate was fed even at the 0.15 mg daily level. Growth on the higher level of feeding was somewhat improved in the cases of 'M' and 'F.'

IV. The results indicate that carotene desorbed from alumina and fed at approximately 0.006 mg. daily retains all its original activity; some activity is lost where silica gel was the adsorbent and only a small amount of activity, if any, is retained by carotene adsorbed on, and then recovered from, ashless norit. Groups V, VI, and VII show the growth response of animals receiving no source of vitamin A, or carotene, or cod liver oil as the sole sources of the vitamin. The growth response of rats receiving as the sole source of vitamin A the feces of either these same negative controls, or carotene-fed, or cod liver oil-fed animals is also shown in these three groups. Since only three of four animals have been used in this study the results may not be significant but there is indication that some carotene or vitamin A activity is lost through excretion in the feces when carotene is fed and that the vitamin A activity of cod liver oil is better utilized. The nutritive condition of the animals receiving the feces of carotene-fed rats was much better than that of the rats receiving the feces of the cod liver oil-fed animals.

carotene recovered from silica gel or from alumina cured every case of xerophthalmia. Unusually good growth and restoration of a good nutritive condition were obtained in the case where alumina had been the adsorbent. In the experiments in which norit had been used as the adsorbent xerophthalmia was never entirely cured, growth was very slow and the animals exhibited muscular incoordination throughout the entire experimental period.

The results with the alumina test material were especially interesting as they show that the physiological activity of the recovered carotene was affected little, if any, by the adsorption process. This indicated that the failure of the test materials to exhibit activity was probably due to the inability of the rat to desorb the carotene from the adsorbent. This contention was borne out by the fact that at autopsy it was observed that the fecal material about to be excreted contained a large amount of the yellow pigment.

In order to study this possibility the feces of rats fed 0.45 mg. daily of carotene adsorbed on alumina, of rats fed cod liver oil, of rats fed crystalline carotene (0.005 mg. daily in ethyl laurate) and of rats receiving no source of vitamin A (negative controls) were collected daily and in each case fed to other vitamin A depleted rats as the sole source of vitamin A. The feces of negative control animals were apparently free of any vitamin A. The three animals fed feces of the cod liver oil-fed rats were little, if any, better than the negative controls. Xerophthalmia was very severe and other infections were found on autopsy. The cod liver oil-fed animals were free from any infection and grew at a rate appreciably greater than that of any of the carotene-fed animals. There apparently was no detectable loss of vitamin A from these animals in the feces. The feces of rats fed 0.005 mg. of crystalline carotene daily, as well as of those fed carotene adsorbed on alumina apparently conveyed some vitamin A activity to the test animals to which they were fed.

SUMMARY

1. The biological activity (vitamin A) of carotene adsorbed on silica gel, on norit carbon, and on alumina has been investigated.

2. It was found that vitamin A deficient rats fed alumina which contained relatively large amounts of adsorbed carotene grew at a rate only slightly below normal but were not completely cured of xerophthalmia. However, rats similarly prepared were apparently unable to utilize adsorbed carotene when silica gel or norit carbon were the adsorbents.

3. The adsorption process did not apparently alter the physiological activity of the pigment when alumina was the adsorbate. Carotene desorbed from silica gel was somewhat less active, while carotene similarly removed after adsorption on ashless norit carbon had lost practically all biological activity.

4. Some evidence is presented which would indicate that the rat does not completely utilize all the carotene fed whether the amount given is large or small. Feces of rats fed large amounts of adsorbed carotene were highly pigmented and the feces of rats fed small amounts (0.005 mg. daily) of carotene in solution were also found to contain vitamin A activity.

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HUMAN MILK STUDIES

XIII. VITAMIN POTENCY AS INFLUENCED BY SUPPLEMENTING THE MATERNAL DIET WITH VITAMIN A ¹

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Animal experimentation has indicated that the concentration of vitamin A in milk is dependent upon its presence in the diet (McCollum, Simmonds and Pitz, '16; Kennedy and Dutcher, '22; Nelson, Lamb, and Heller, '22). Kennedy, Palmer, and Schlutz ('23) reported that 10 cc. of breast milk daily from women on an adequate dietary met the vitamin A requirements of rats, but that milk from women on diets deficient in this substance did not. In this laboratory daily intakes of 2.5 and 3 cc. of pooled milk from a group of wet nurses on average American dietaries were found to satisfy the vitamin A needs of both growth and reproduction of the rat (Macy et al., '27). Conversely, similar quantities of milks of two women with high production records tested singly in a similar manner contained a lower amount of vitamin A, although the maternal diets were apparently satisfactory (Macy et al., '28).

These observations on the biological potency of breast milk become more significant when considered in the light of the recent study of Thatcher and Sure ('32) in which they report

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² Formerly the Nutrition Research Laboratories of the Merrill-Palmer School and the Children's Hospital of Michigan.

autopsy findings of a case of avitaminosis in an infant who had been fed exclusively by breast milk from a starving mother. That the infant had been suffering from a lack of vitamin A in the diet before its admittance to the hospital was demonstrated by the presence of bilateral ophthalmitis with a left corneal ulcer 5 mm. in diameter, the outward manifestation of an acute nutritional upset which has been present for some time, and in spite of the administration of a diet containing cod liver oil during the last 14 days of life death occurred from bronchopneumonia. Post-mortem findings were those typical of vitamin A deficiency.

In view of the variability in the vitamin A concentration of mother's milk and its dependence upon her diet and subsequent availability of vitamin A from her body tissues and through breast milk to satisfy the particular physiological needs of growth of the infant, it is of clinical significance to determine whether breast milk can be enriched by supplementary additions of the maternal dietary with a concentrated source of this substance. For this purpose three women each producing large but different quantities of milk expressed all of their milk daily and besides feeding their babies by bottle were able to send sufficient milk to the laboratory for the biological tests.

The pre-cod liver oil studies on the vitamin A content of the separate milks produced while the three women were on their accustomed home diets were begun 6 weeks post-partum after mature milk flow had been established and extended to the sixth month. After these preliminary tests were complete each woman then took daily 15 gm. of cod liver oil³ in gelatin capsules for 1 month preceding and throughout the seventh to the ninth months of observation to determine if vitamin A is transferred quantitatively into the milk from the dietary adjuvants. The quantity of milk secreted daily by each woman remained practically constant throughout the period of observation, namely, 3134, 2366, and 1419 cc. daily

³ We wish to express our thanks and appreciation to A. D. Emmett, Ph.D., and E. A. Sharpe, M. D., of Parke, Davis & Company, Detroit, for the cod liver oil used in this study.

with average fat contents during the former period of 3.9, 3.8, and 4.8 gm. per 100 cc., respectively and during the period of cod liver oil consumption of 4.0, 3.6, and 5.5, respectively (Nims et al., '32). The breasts were completely stripped each morning, the milk being allowed to flow directly into a glass bottle, then sealed air tight, and packed in ice until it was fed 2 hours later to the test animals. A technic previously standardized for these studies and reported elsewhere (Macy et al., '27) was used for the biological testing of the milk. Quantities of $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, and $2\frac{1}{2}$ cc. of each milk were fed daily to the experimental animals apart from the basal diet as the sole source of vitamin A. Weekly body weight and food consumption records were made. The results were recorded in average gain of all animals receiving a particular quantity of an individual milk. In addition, the time of the opening of the vaginal orifice was noted and vaginal smears were examined daily thereafter for the detection of persistent cornified epithelial cells, one of the earliest symptoms of vitamin A exhaustion in the body (Evans and Bishop, '22).

From the average gain of groups of test rats fed quantities of $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, and $2\frac{1}{2}$ cc. daily of the individual breast milks as the only source of vitamin A it was evident that as the quantities of each milk were increased the growth and physiological well-being of the experimental animals responded proportionately. One-half cubic centimeter of milk daily was insufficient for satisfactory growth, but as the quantities were augmented improvement occurred with each succeeding increase until nearly normal growth with the entire disappearance of all symptoms of avitaminosis and complete return of the normal oestrous cycle was secured with $2\frac{1}{2}$ cc. of milk daily. The total food intake of rats expressed in calories per square meter of surface area per 24 hours was not a determinate factor. As judged by the average gain in body weight of the experimental rats and the vaginal smear records, the vitamin A content of the individual milks from the three women irrespective of volume flow of milk and fat contents was essentially the same and remained unchanged during the

period of supplementary additions of 15 gm. of cod liver oil daily to an abundant and well-chosen dietary.

These findings recorded on the inability of three women to transfer increased quantities of vitamin A to their milk beyond the usual potency on an adequate dietary when daily supplementary additions of vitamin A were made to the maternal diets are in accord with those of Moore ('31, '32) who found that in the cow the vitamin A and carotene of the butterfat are maintained at normal values in spite of large dietary excess. Dann ('32) made a similar observation on rats and rabbits, stating, "There must be some factor at work limiting quite strictly the amount of vitamin A which can pass through the placental barrier or into the milk." Of additional interest is the study of Vogt ('32) who found that the vitamin A content of tissues obtained from women at operation show that the subcutaneous fat tissue is an important place of storage of vitamin A, the concentration being greatest in the mammary gland. The parenchyma of the mammary gland itself may have the function of controlling the maximum amount of vitamin A that passes into the milk.

These studies on the transmission of vitamin A are paralleled by similar ones on vitamin B. Gunderson and Steenbock ('32) found that increasing vitamin B intake both absolutely and in relation to the amount of milk secreted had no discernible effect on the vitamin B content of cow's and goat's milk and they suggest that the maximum vitamin B content is under definite physiological control. Observations on women in this laboratory corroborate the above findings (McCosh et al., '31; Donelson and Macy, '34). In studying milks of different women before and after supplementing their usual diets with 10 gm. of yeast daily it was found under the conditions of the experiments that the vitamin G potency of the breast milk was increased but there was no appreciable change in the vitamin B concentration.

In face of the fact that mother's milk produced during inadequate dietaries may fall short of meeting the optimal concentration of vitamin A in the milk and infants may fail

to secure adequate amounts of this factor for optimal growth and storage, clinicians can well afford to see that their nursing mothers receive in their diets a sufficient amount of this important food substance to maintain its maximum values in breast milk and thereby benefit both the mother and her child.

SUMMARY

The vitamin A content of the individual milks for three women remained essentially the same before and during a period of supplementary additions of 15 gm. of cod liver oil daily to an abundant and well-chosen diet.

The results of the biological assays are based upon the average gain in body weight of the experimental rat and on the vaginal smear records. It is pointed out that mother's milk, produced during an inadequate dietary regime, may fall short of meeting the optimal concentration of vitamin A in the milk and therefore the physiological needs of the infant.

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ANTERIOR PITUITARY GROWTH HORMONE AND THE COMPOSITION OF GROWTH

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ONE FIGURE

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One of the steps to be taken in the study of the physiologic mechanisms through which the growth hormone of the anterior pituitary acts is the determination of the character and composition of the growth which is effected. If the increase in weight commonly obtained by the injection of potent extracts is due merely to the addition of water or fat, it can hardly be regarded as true growth, which must be considered to involve essentially an increase in the mass of body protoplasm, with protein synthesis a necessary accompaniment. Between the extremes of dwarfism produced by removal or destruction of the anterior lobe and giantism produced by hyperactivity, there is no question that true growth in the latter sense is stimulated or conditioned by the hormone. The stimulation, however, may involve neither a general effect upon anabolic processes, nor any specific effects upon metabolic mechanisms of the body for the synthesis and deposition of particular constituents. It may be facilitated by or entirely dependent upon such factors as appetite, digestibility and the amount of food consumed, and act by causing a plethora of nutritive elements to be available for ordinary processes. That these factors are of real significance is indicated, among other things, by some pertinent evidence of Osborne and Mendel and their associates. For example,

Anderson and Smith ('32) have been able to produce giant rats, rivaling in size those obtained by treatment with anterior pituitary extracts or grafts, merely by the feeding of particularly favorable diets.

If the excess growth caused by the hormone is partially or wholly independent of these factors and is not solely due to the character of the diet or to an increased food consumption then it is reasonable to look for specific influences upon particular metabolic mechanisms or for some property of the principle necessary for the anabolic use of the available food constituents.

In the present investigation we have determined the effects of the growth hormone upon the gain in weight and upon the composition of that gain in rats, with the factor of the amount of food consumption carefully controlled. In order to realize this control, the 'paired-feeding' method, as highly developed and analyzed by Mitchell ('30) was chosen. This method would appear to be peculiarly suitable for studies of endocrine effects upon nutrition. It is not necessary to feed rather artificial purified diets from which certain constituents under study have been carefully excluded and upon which the best growth obtainable may not be very great. On the contrary, complete diets adequate for normal growth or better, can be used advantageously, provided they do not contain in an assimilable form the hormone under study.

EXPERIMENTAL PROCEDURES

The rats were selected as pairs, triplets or quadruplets. In each set they were litter-mates of the same sex and of nearly the same body weight and body length at the beginning of the experiment. Upon unrestricted feeding they had also demonstrated equal abilities for growth since weaning. In addition, they were so distributed in the two experimental groups as to give those which were to serve as controls whatever slight initial advantage there was in individual weight, length and past growth over those which were to receive the growth hormone. The ages ranged from 52 to 224 days.

One animal of each pair, trio or quartet was chosen for a control and a mate to be treated. The third rat of each trio, designated as a 'check,' was killed at the beginning of the experimental period. Its analyzed composition was assumed to be the composition of its two mates at that time. Of each quartet, one animal became a control, one a treated, one a check and the fourth was chosen to be a treated rat fed *ad libitum*. There were two sets of pairs, eight sets of triplets and two sets of quadruplets. The check rats and their control and treated litter-mates are correspondingly numbered in the tables. In addition, check rat 6 was the trio mate of pair 11, and check rat 7 was the trio mate of pair 12. Two pairs, 9 and 10, had no check mates for analysis; their initial composition was calculated from the average composition of check rats 2, 3, 4, 5 and 6. The two treated rats fed *ad libitum* were included in order to determine directly the influence of the hormone upon absolute appetite and the maximal growth which could be secured with conditions the same except as to the amount of the food consumption.

In figure 1, B and C are the composite growth curves of ten of the rats selected for treatment and their ten control mates for 7 weeks before the beginning of the experimental period. Feeding during this time had been unrestricted and the two groups had maintained essentially the same growth rates, with those destined to be the controls a few grams heavier each week. Two pairs of animals were too young to be included in these curves. The composite growth curve of seven of the set mates selected for carcass analysis at the beginning of the experiment is shown in A.

The diet used commonly gives growth rates exceeding those of Donaldson and of King ('24). Its composition was: whole ground wheat, 49.7 per cent; yellow corn meal, 6.0 per cent; G.L.F. calf meal, 7.0 per cent; casein, 12.0 per cent; skim milk powder, 16.0 per cent; fat (lard, butter, corn oil, cod liver oil concentrate), 4.0 per cent; salt mixture (Hawk's modification of Osborne and Mendel's), 4.3 per cent; Harris yeast concentrate, 'Bemax' and lemon juice, 1.0 per cent. The food was sifted, the screenings reground and the whole batch

thoroughly mixed in a Hobart machine. The mixed food was sealed in 1-pound cans until needed. Analyses of the food

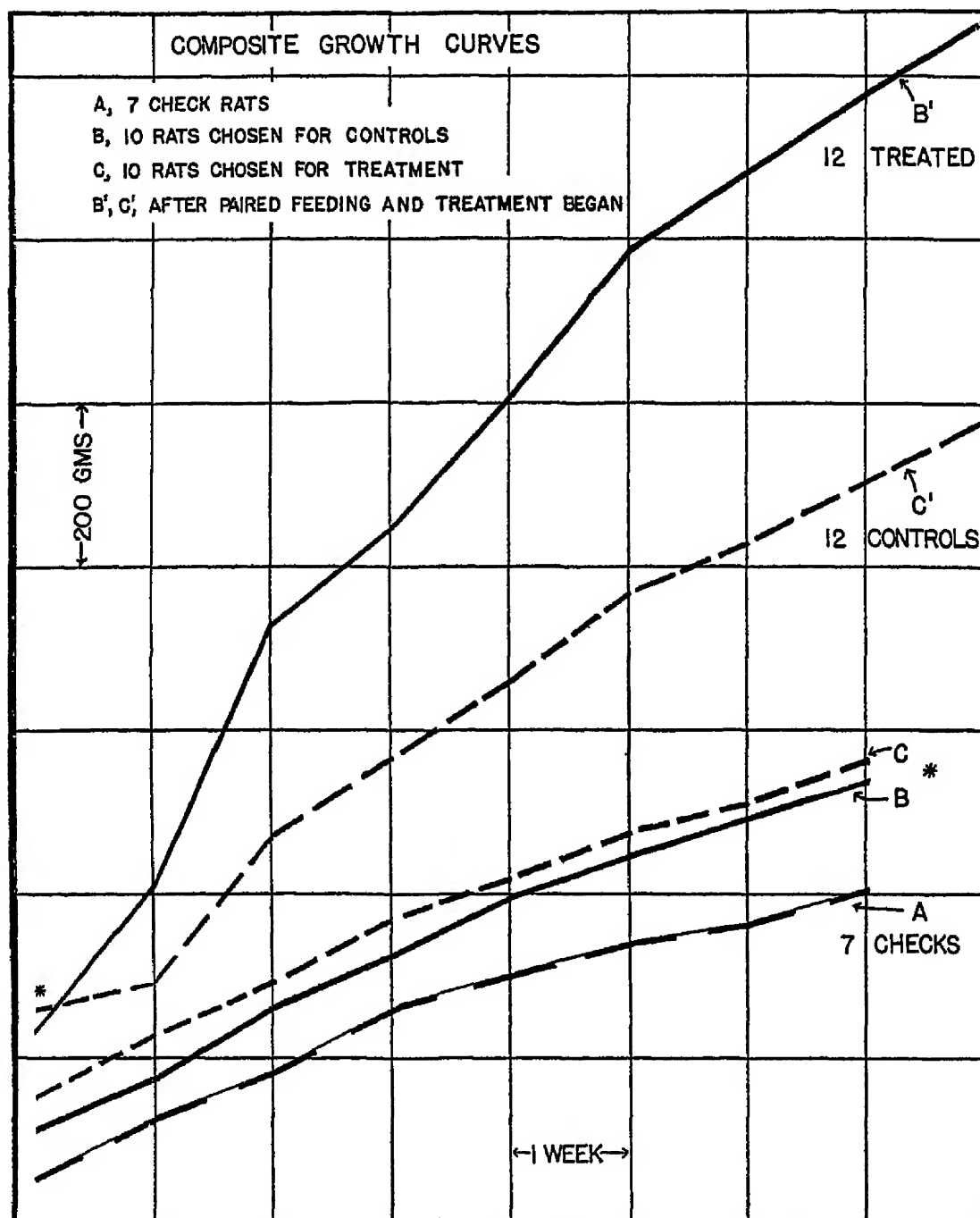


Fig. 1 Curves B' and C' are continuations of curves B and C after the beginning of paired-feeding and treatment, and with the addition of two pairs of animals.

showed its composition to be: water, 8.4 per cent; fat, 5.6 per cent; ash, 6.4 per cent; nitrogen, 3.7 per cent; carbohydrate (by difference), 56.5 per cent; and energy value, 4.20 Calories per gram. The food was fed in 35 cc. porcelain evaporating

dishes inserted into receptacles attached to the outside of the cages. These receptacles were made from 8-ounce ether cans, provided with a metal apron which covered about one-fourth of the food dish and which extended about 3 cm. inside the cage. The opening was of such size and the apron was at such an angle that a rat could get its head but not its feet into the food dish. As further precaution against wastage the food was moistened to a thick paste with water. Particular search was made each day for uneaten food carried out into the cage, and on only one occasion was any evidence found of such wastage. The rats were confined in individual wire cages 12 inches in diameter and 6 inches high with screen bottoms.

At each daily feeding the treated and control mates of each pair were given the same quantity of food. This amount was so adjusted that the less hungry member of the pair usually left about 1 gm. of its ration uneaten. Each animal's food was weighed daily to 100 mg. from its individual weighed flask. On the last day of each week the amount fed was diminished a few grams so that even the less hungry mate would consume all that was offered, and the total intake for the week was equalized by weighing the individual flasks to 5 mg. The amount of food eaten by each group of twelve animals was 9766 gm. in the whole period of 763 days, or an average of 12.8 gm. per rat per day. Water was allowed at will, but for eight pairs the amounts consumed weekly were measured. The length of the experimental period of paired-feeding and treatment before killing for carcass analyses was 8 weeks for five pairs, 9 weeks for three pairs, 10 weeks for two pairs and 11 weeks for two pairs.

For eight pairs of animals and two *ad libitum* feeders, collections of urine, feces and shed hair were made. For this the cages were set into paraffin-coated tinned funnels with removable brass-wire screens. Feces were removed daily and the whole cage bottom and funnel thoroughly washed down with hot $\frac{1}{2}$ per cent acetic or hydrochloric acid. The use of a spray gun permitted very thorough washing without diluting the urine too greatly. The urine collections were pooled weekly for each group of rats, and samples were preserved in an ice box with small amounts of chlor-thymol. Feces were collected for the whole period, dried to constant weight at 60°C., finely ground, thoroughly mixed, and again dried. The shed hair and the alimentary tract contents at the time of killing were collected, weighed, dried and analyzed separately.

Standard procedures and methods for chemical analyses were used. Each rat was killed with illuminating gas, and the alimentary tract was stripped of its contents. The body, including the emptied gut (hereafter called the 'empty carcass' and its weight 'E. C. W.') was ground and dried to constant weight at 60°C. Fat of the hashed and dried empty carcass was thoroughly extracted with petroleum ether in a Soxhlet apparatus. The fat-free dry tissue was pulverized, mixed and dried again. Samples of this powdered tissue were taken for nitrogen, ash and calorific value determinations. The ether extract after evaporation of the ether, was analyzed for the small amount of nitrogen and ash which it contained, and its heat of combustion was determined. For nitrogen, the macro-Kjeldahl method was used; for ash, incineration in a muffle furnace; for heat value, samples were burned in the oxycalorimeter of Benedict and Fox ('25). The same methods were used for the analyses of urine, feces, shed hair, alimentary tract contents and food, except that for the calorific value of urine the figure 8.09 Calories per gram of nitrogen was assumed (Benedict and Milner, '07). The determinations on ash are the least accurate of the analyses. The average difference in duplicate ash determinations on all tissue samples was 2 per cent, and the absolute values for tissues are somewhat low because of the method of incineration used.

The anterior lobe extract used was made by ourselves from fresh beef pituitary glands.¹ It was a purified, assayed preparation, highly potent in stimulating growth in either hypophysectomized or normal assay rats. The amount injected subcutaneously each day corresponded to three rat units by our assay (unpublished results), or between one-half and three-fourths of the least amount which will cause approximately maximal growth response in rats under the assay conditions. The amount corresponded also to the growth-promoting potency of from 40 to 60 mg. of fresh beef anterior lobe. A similar but less purified extract of ours was found by Riddle ('32) with his highly sensitive pigeon test to be free of the lactation principle and to have only traces of the gonad-stimulating principle. By the less sensitive rat test

¹We are indebted to Mr. Frederick Fenger and Armour & Co., Chicago, for supplying us with fresh pituitary glands, shipped packed in CO₂ snow.

and much larger doses of the extract used in these experiments we could not detect any gonad-stimulating activity.

RESULTS

Weight. As shown by the composite growth curves B' and C' in figure 1, the twelve treated animals immediately passed their control mates in weight after paired-feeding and treatment with the hormone were begun, and continued to show an

TABLE 1
Weekly excess gains (in grams) of treated rats over controls

PAIR NO.	WEEKS											TOTAL
	1	2	3	4	5	6	7	8	9	10	11	
1F	8	12	8	12	3	7	11	-4	8	10		75
2F	14	11	-2	12	8	4	0	1	0			48
3F	15	19	5	0	3	3	-7	2				40
4F	20	19	6	7	4	5	8	-2	4			71
5F	19	16	9	7	8	8	0	-2				65
6F	13	14	-4	11	3	10	-3	-4				40
7M	-3	-5	-3	5	0	-6	-7	4	2	-3	6	-10
8M	8	-9	6	14	9	5	30	3	0			66
9F	9	14	1	8	14	5	6	5	-10	3		55
10F	21	6	10	-5	5	4	-4	-3				34
11F	15	15	0	11	2	6	0	2				51
12M	4	1	3	-5	5	-10	-4	7	5	0	2	8
Totals	143	113	39	77	64	41	30	9	9	10	8	543
Ad lib. feeders												
2AF,												
3AF	40	33	27	9	19	6	7	7				148

excess gain each week to the end of the experiment. The curves in figure 1 are plotted only to the eighth week since at that time five pairs were killed for analysis.

The distribution of the excess gains of the treated rats by weeks and by pairs is given in table 1. At the end of the whole period eleven of the twelve had shown an excess gain in weight over their control mates on the same food intake, amounting to 543 gm., or equivalent to the weight of two extra adult animals. The means of the excess gains by the treated

animals were 5.0 gm. per rat per week and 45.3 gm. per rat for the whole period. On the assumption that, if the growth hormone were without effect, the chances are even that either rat of any pair would gain more than its mate, the observed outcome favoring the treated animal in eleven of twelve instances would be expected to occur by chance once in 341 times. With a probability of this order it is obviously unnecessary to carry the statistical analysis further, either by consideration of the magnitude of the total excess gains or of the 109 weekly comparisons of individual gains.

Treated rat, 7TM, which gained 10 gm. less than its control mate and another rat, 12TM, which showed only 8 gm. excess gain, were males from the same litter and were only 52 days old when the experiment began. They failed to show any excess gain from the first, but they and their controls were already growing rapidly. At the time they were killed their growth rates were still high and they were perhaps just approaching their plateaus. The other male rat, 8TM, did not show much excess gain at first, but apparently became more responsive as the treatment continued. That male rats are relatively more refractory to the growth hormone has been noted by Evans and Simpson ('31), and occasionally a whole litter, both males and females may be partially resistant. Rat 7TM, despite its negative response as judged by body weight, showed a positive effect in the composition of its gain. It added 1.1 gm. of nitrogen, 4 gm. of water and 0.9 gm. of ash more than did its control mate, and 10.7 gm. less of fat. Rat 12TM showed as anomalous a response in its composition as in its weight. It gained an insignificant excess of nitrogen, 0.5 gm., the same amount of ash and 10 gm. more of fat. Its excess gain in body length, 0.9 cm., is probably significant.

Notwithstanding the dilution which the inclusion of these two young males gives, the effect of the hormone on the gain in weight of the treated group as a whole was considerable. These twelve animals gained 1295 gm., or 55 per cent, of their initial live weight. Their twelve controls gained 764 gm., or 32 per cent, of their initial weight.

Body length, nutritive ratio and surface area. The average percentage increase in body length was 7.4 for the control and 13.5 for the treated rats. For each group this was only about one-fourth as much as the average percentage increase in weight. This, however, does not indicate the development of any abnormalities in body shape. Even the excess growth of the treated animals was not disproportional, as indicated by their nutritive ratios. This ratio $\frac{W^{1/3}}{L}$, gives a good measure of body shape and reflects disproportions between weight and length. The average ratios for the check, and for the control and treated rats at the beginning were 0.284, 0.283 and 0.283, respectively, with standard deviations of ± 0.004 . For both the control and treated rats at the end of the experiment, the average ratio was 0.290 (S.D. ± 0.005). Such an increase in the ratio is about what would be expected in normal well-nourished rats passing the plateaus of their growth curves, and it is only surprising that the more rapidly growing treated animals did not show a greater elongation of the trunk relative to the linear dimension of body weight than did the controls.

The sums of the surface areas, calculated from body weights and lengths (Lee and Clark, '29) at the beginning of the experiment were 3528 sq.cm. for the twelve controls and 3517 sq.cm. for the twelve treated. The controls gained a total of 562 sq.cm., or 16 per cent, and the treated 991 sq.cm., or 28 per cent, an excess over the controls of 429 sq.cm., or 12 per cent, of their initial area.

Appetite. An opportunity was afforded by the technic of the paired feeding method to compare the relative appetites of control and treated rats. The results were usually indeterminate for the last day of each feeding week because that day's ration was cut for each rat so that even the less hungry member would eat all of its allowance. If both rats of a pair left approximately the same amounts of food they were considered to be equally hungry.

The appetite of each rat was determined on unrestricted feeding for a few days before the beginning of the paired-

feeding. Of 30 such determinations the rats destined to be controls were more hungry 18 times and those to receive treatment 10 times. This might be expected from the fact that the controls were slightly heavier than their mates. During the course of the experiment in 763 daily instances, the control rats were more hungry 91 times, 50 of which were in the first week; the treated rats were more hungry 555 times, 31 of which were in the first week; both rats were equally hungry 10 times; and in 107 instances the result was indeterminate. Hence, after the first week, in only 46 of 575 determined instances were the controls more hungry than the treated.

The conclusion is confirmed by direct observation and by the food consumption of the two rats fed *ad libitum*. The treated rats ate the most of their daily food allowance soon after it was supplied to them. Often the entire amount was eaten within 3 to 4 hours. The controls, on the other hand, usually did not consume much or any of their food until night. The amounts consumed by the two treated rats fed *ad libitum* were 824 and 741 gm. in the 63 and 56 days they were on the experiment, or 13.2 gm. per rat per day. This was an average excess of 1.6 gm. per day, or 13 per cent, over their mates on paired-feeding.

Water consumption. The water intake was not restricted, but the amount that was drunk was measured for the eight control and eight treated animals used for urine and feces collections. Each of these groups in 504 days drank practically the same amounts, 5.6 kg. This was in addition to the approximately equal amounts they received in the moistened food.

Activity and behavior. Unfortunately, no quantitative records could be made of the voluntary muscular activity of the animals. Incidental observations, however, gave the impression that the treated animals were more alert and active than their controls. The consumption of much of their daily rations immediately after feeding has been mentioned. They also usually cleaned their food dishes thoroughly, whereas the

controls commonly left dried particles adhering to the edges where access was difficult. The controls usually slept the greater part of the day, but the treated rats were easily aroused to such activities as gnawing at the wire cages and the metal food-dish holders. They were decidedly less tame to handle than the controls.

Composition of the animals and of the gains. The compositions of the individual check rats are given in table 2, and of the treated and controls, determined at the end of the experimental period, in table 3. Several considerations indicate the validity of the assumption that the initial compositions of the two groups on paired-feeding can be estimated

TABLE 2

Data on check rats, killed at beginning of experiment, and composition of carcasses without alimentary tract contents

NO AND SEX	AGE	BODY LENGTH	LIVE WEIGHT	EMPTY CARCASS WEIGHT	COMPOSITION, BY WEIGHT AND PERCENTAGE, OF EMPTY CARCASS				ENERGY
	Days	Cm.	Gm	Gm	Water	Ether ext.	Total N.	Ash	Cal./gm. E.O.W.
1ChF	101	18.6	148	137.7	85.1 61.8%	18.0 13.1%	4.32 3.14%	5.99 4.35%	2.35
2ChF	101	19.4	158	150.7	94.5 62.7%	16.1 10.7%	5.00 3.32%	7.42 4.92%	2.20
3ChF	153	20.3	177	165.5	105.9 64.0%	13.3 8.0%	5.84 3.53%	7.95 4.80%	2.01
4ChF	107	20.4	207	197.1	116.8 59.3%	32.9 16.7%	6.19 3.14%	7.89 4.00%	2.65
5ChF	159	21.6	250	244.5	142.8 58.4%	43.9 18.0%	7.61 3.11%	10.45 4.27%	2.75
6ChF	171	21.0	206	201.3	122.6 60.9%	27.4 13.6%	6.56 3.26%	8.92 4.43%	2.43
7ChM	53	19.0	172	155.4	104.6 67.3%	12.6 8.1%	4.96 3.19%	5.85 3.76%	1.87
8ChM	224	23.4	293	280.7	174.7 62.2%	31.0 11.1%	9.87 3.52%	11.60 4.13%	2.24
Totals	1069	163.7	1606	1533.0	947.0 61.8%	195.3 12.8%	50.35 3.28%	66.07 4.31%	2.34

TABLE 3
Data on control and treated rats at beginning and end of experiment, and analysed composition of empty carcasses at end

NO. AND SEX	AGE, DAYS		BODY LENGTH, CM.		LIVE WEIGHT, GM.		EMPTY CARCASS WEIGHT, GM.	COMPOSITION OF EMPTY CARCASS AT END				
	Begin	End	Begin	End	Begin	End		Water, gm.	Fat, gm.	Nitrogen, gm.	Ash, gm.	ENERGY, Cal.
1CF	101	171	18.5	19.7	143	188	180	105	33.6	5.37	7.6	502
1TF	101	171	18.6	22.2	142	262	250	165	21.7	8.23	10.2	489
2CF	101	164	19.3	20.5	153	202	193	111	36.8	5.96	8.2	550
2TF	101	164	19.2	21.3	156	252	241	140	44.4	7.59	9.2	673
3CF	153	209	20.2	20.8	183	211	200	114	37.4	6.43	9.3	572
3TF	153	209	20.4	22.1	186	250	236	135	41.7	7.50	10.5	659
4CF	107	170	20.2	21.9	200	247	236	130	52.3	7.19	9.8	737
4TF	107	170	20.3	23.1	195	312	295	186	34.6	9.70	12.9	659
5CF	159	215	21.9	22.6	252	282	267	147	58.3	8.07	11.2	828
5TF	159	215	21.8	24.0	250	344	320	198	40.8	10.62	13.3	749
6CF	171	227	21.0	21.3	209	222	208	122	35.1	6.74	9.0	563
6TF	171	227	20.9	22.0	205	256	239	150	26.8	7.73	11.1	534
7CM	52	129	19.0	24.2	166	373	354	212	71.8	8.89	11.6	991
7TM	52	129	18.9	24.2	168	364	356	217	61.3	10.04	12.5	925
8CM	224	287	23.4	23.8	290	324	305	188	44.1	9.78	12.8	748
8TM	224	287	23.6	25.8	290	390	369	241	35.9	12.06	14.6	753
9CF	145	215	21.0	21.3	230	273	265	143	68.4	6.83	9.0	885
9TF	145	215	21.2	23.3	228	325	314	204	34.1	9.69	13.5	664
10CF	135	191	20.8	21.3	207	228	220	123	44.4	6.76	9.9	655
10TF	135	191	20.9	22.9	203	258	248	160	22.5	8.75	11.0	509
11CF	171	227	21.0	21.3	209	220	213	122	37.0	6.79	9.3	588
11TF	171	227	20.8	22.1	201	264	248	161	22.3	8.35	10.5	504
12CM	52	129	18.6	24.4	140	376	363	227	52.5	11.01	13.2	872
12TM	52	129	18.2	24.9	134	376	365	217	62.7	11.30	13.0	973
Totals control	1571	2334	245	263	2382	3146	3004	1743	572	89.8	120.8	8490
Totals treated	1571	2334	245	278	2358	3653	3481	2175	449	111.6	142.2	8091

with a sufficient degree of accuracy from the analyses of their check mates. The only limitation of the assumption is the normal variation, which should be at a minimum among litter-mate rats of the same sex, weight and previous growth history. Although enough data are not available to give a statistical assessment of these limits, the analyses published by Mitchell and Carman ('26) indicate that the variability is less for litter-mates than for a random selection of rats of about the same age. The litter-mates of their series, how-

TABLE 4
Compositions of check, control and treated rats

GROUP	EMPTY CARCASS WEIGHT, GM.	AVERAGE COMPOSITION OF EMPTY CARCASS					
		Water	Ether ext.	Fat free dry tissue	Nitrogen	Ash	Energy Cal /gm
12 checks	2273	62.0%	12.7%	25.3%	3.25%	4.29%	2.33
S.D. \pm		2.64	3.14	1.25	0.147	0.353	0.267
P.E. \pm		0.51	0.61	0.24	0.029	0.069	0.052
C.V. \pm		4.4%	24.8%	4.9%	4.5%	8.2%	11.4%
12 controls	3004	58.0%	19.1%	22.9%	2.99%	4.02%	2.83
S.D. \pm		2.54	3.10	1.57	0.225	0.420	0.257
P.E. \pm		0.50	0.60	0.31	0.044	0.082	0.050
C.V. \pm		4.4%	16.3%	6.8%	7.4%	10.4%	9.1%
12 treated	3481	62.5%	12.9%	24.6%	3.20%	4.09%	2.32
S.D. \pm		2.80	3.61	1.33	0.168	0.337	0.301
P.E. \pm		0.54	0.70	0.26	0.033	0.066	0.059
C.V. \pm		4.4%	28.0%	5.4%	5.2%	8.2%	13.0%

ever, differed as much as 10 per cent in weight. Nitrogen, ash and fat-free dry tissue formed fairly constant proportions of the empty carcass weight, even among different litters and with wide ranges of body weight. This is also true of our series, as shown in table 4, by the coefficients of variability from the means of the constituents determined in check and control rats.

If the data both for the rats analyzed by Mitchell and Carman and for the check and control rats of the present series are plotted as scatter diagrams, there are found to be

very high correlations between body weight or body length and the content of nitrogen, fat-free dry tissue, ash or water, in about the order listed. The constituents least correlated with weight or length are fat and energy. Since initially each trio of rats showed insignificant differences in body weight and length, these high correlations indicate that the discrepancy between estimated and true composition must have been small for all constituents except possibly fat.

As is well known, fat is the most variable constituent of an animal's carcass, and tends to increase with age (Moulton, '23). The coefficients of variation for ether extract in the present series of rats are three to eight times greater than those for any other constituent. As shown in table 5, the control rats at death contained almost twice as much fat as they had initially, although they had gained only 32 per cent

TABLE 5

Summary of total gains and composition of gains in control and treated rats

	BODY LENGTH, CM.	LIVE BODY WEIGHT, GM.	EMPTY CARCASS WEIGHT, GM.	COMPOSITION OF EMPTY CARCASS WEIGHT GAIN, GRAMS AND PER CENT					ENERGY, CAL
				Water	Ether ext.	Fat-free dry tissue	Total N.	Ash	
12 controls									
Total gain	18.2	764	716	324	281	111	15.4	22.7	3161
Per cent of initial datum	7.4	32.1	31.3	22.8	96.7	19.2	20.7	23.1	59.3
Per cent of gain in E.C.W.				45.2	39.3	15.5	2.15	3.16	4.41 ¹
12 treated									
Total gain	33.1	1295	1217	771	162	284	37.9	45.1	2820
Per cent of initial datum	13.5	54.9	53.7	54.9	56.3	49.6	51.5	46.4	53.5
Per cent of gain in E.C.W.				63.3	13.3	23.4	3.12	3.71	2.32 ¹
Excess gain of treated	14.9	531	501	447	-119	173	22.5	22.4	-341
Per cent of initial datum	6.1	22.5	22.1	31.8	-41.5	30.2	30.6	23.0	-6.5
Per cent of excess E.C.W. gain				89.2	-23.8	34.5	4.49	4.47	-0.68 ¹

¹ Calories per gram of empty carcass weight.

of their initial weight. The percentage of water tended to decrease with increase in fat. The energy content reflects the changes in fat and was the second most variable constituent.

Total nitrogen formed a remarkably constant proportion of the fat-free dry tissue, ranging from 125 to 132 mg. per gram for all three groups of check, control and treated animals. The nitrogen content per gram of fat-ash-free dry tissue ranged from 15.1 to 16.3 per cent for the whole group of thirty-two check, control and treated animals analyzed. The mean was 15.6 per cent with a standard deviation of ± 0.33 . This is slightly lower than the commonly accepted value of 16 per cent, probably because the ash values are somewhat low.

The water content closely paralleled the nitrogen content of the carcass. The ratios of water to total nitrogen were: check rats, 19.1; controls, 19.4; treated, 19.5; gain of controls, 21.0; gain of treated, 20.4. The ratios of ash to total nitrogen were also fairly constant: checks, 1.31; controls, 1.34; treated, 1.28; gain of controls, 1.47; gain of treated, 1.19.

The most striking finding as to composition was the fact that the treated rats retained almost exactly their initial average percentage composition for all constituents and for energy per gram of empty carcass weight. All of the differences were less than their probable errors, except the difference in the percentage of ash, which was only twice its probable error. As this would indicate and as shown in table 5, the gain by the treated rats for every constituent was in the same proportion as the gain in empty carcass weight—around 50 per cent of the initial datum. This means, too, that the composition of the gains in weight as given in table 5, was closely similar to the composition of the rats themselves. The controls, on the other hand, showed the characteristic changes with age that would be expected in well-nourished rats after puberty and reaching the plateaus of their growth curves—relative decreases in the proportions of water, nitrogen and fat-free dry tissue, and increases in fat and energy. These differences were, respectively, 5.6, 4.9, 6.2, 7.3 and 7.0 times

their probable errors, which indicates significance notwithstanding the slight uncertainty as to the estimated initial composition. The decrease in the percentage of ash was only 2.5 times the probable error of the difference and is perhaps not significant.

The differences in percentage composition between the control and treated rats at the end had the following ratios to their probable errors: water, 6.1; fat, 6.6; nitrogen, 4.0; ash, 0.7; fat-free dry tissue, 5.8 and energy, 6.6. These ratios indicate high significance for all differences except ash, and are based entirely upon analyzed compositions. The number

TABLE 6

Statistical analysis of excess gains of twelve treated over twelve control rats

VARIABLE	MEAN EXCESS GAIN OF TREATED GROUP	t (FISHER)	P
Body length	1.24 cm.	7.18	< 0.01
Live weight	44.25 gm.	5.92	< 0.01
Empty carcass weight	41.75 gm.	6.65	< 0.01
Fat-ash-free dry tissue	12.58 gm	7.86	< 0.01
Total nitrogen	1.88 gm	7.81	< 0.01
Water	37.25 gm	5.67	< 0.01
Ash	1.87 gm	5.23	< 0.01
Ether extract	-9.98 gm.	-2.74	< 0.02
Total energy	-28.45 cal	-0.99	< 0.35

of animals in each group was small, however. Since the true value of the standard deviation may only be roughly approximated in small samples, a more rigorous method of statistical treatment would be the determination of 't' and P values for Fisher's ('30, pp. 107 and 139) measure of significance for the difference in the means of small samples. These values for the difference in mean gains of treated and control groups, for the several variables are given in table 6. The probability values (P) indicate high degrees of significance except for total energy. The differences in percentage composition of control and check rats, analyzed by the same method, are all highly significant except for the percentage of ash. The differences in the percentage composition of the

treated and the check rats are entirely insignificant for all variables.

Nitrogen and ash balances. The nitrogen and ash balances, as determined in eight pairs, are shown in table 7. Of the total nitrogen intake, 3.5 per cent for the controls and 4.8 per cent for the treated was apparently lost in collection, perhaps as ammonia from the urine. For ash, on the contrary, more was recovered in urine, feces, and carcass gain than was consumed. This is in part accounted for by the fact that the acid used in washing the funnels and brass screens dissolved some metal. The retentions of both nitrogen and

TABLE 7
Summary of nitrogen and ash balances

GROUP	INTAKE	EXCRETION AND WASTAGE				RETEN- TION BALANCE	RETENTION, TISSUE ANALYSIS	DIFFER- ENCE
		Urine	Feces	Shed hair	Total			
I. Nitrogen								
(A) 8 controls								
Grams	234.8	185.3	31.1	1.8	218.3	16.5	8.3	8.2
Per cent of intake		78.9	13.3	0.8	93.0		3.5	3.5
(B) 8 treated								
Grams	234.3	171.6	25.3	2.7	199.6	34.7	23.5	11.2
Per cent of intake		73.2	10.8	1.2	85.2		10.0	4.8
Difference (B-A)								
Grams		-13.7	-5.8	0.9	-18.7	18.2	15.2	3.0
II. Ash								
(A) 8 controls								
Grams	405.6	202.6	198.0	2.5	403.0	2.6	13.7	11.1
Per cent of intake		50.0	48.8	0.6	99.4		3.4	2.7
(B) 8 treated								
Grams	405.0	197.0	186.0	3.1	386.2	18.8	28.7	9.9
Per cent of intake		48.6	45.9	0.8	95.4		7.1	2.4
Difference (B-A)								
Grams		-5.6	-12.0	0.6	-16.8	16.2	15.0	-1.2

ash, determined directly by carcass analysis, are far more reliable than the retentions determined indirectly from the balance between intake, excretion and wastage. The excess retentions by the treated rats, however, show close agreement by the direct and indirect methods of determination, indicating that the errors of collection were approximately the same for the two groups.

The weekly excess urinary nitrogen excretions by the eight controls over their treated mates for the first 8 weeks of the experiment were serially: 4.2, 3.8, 0.2, 2.7, 0.9, 1.5, 0.9 and -1.1 gm. The weekly excess retention by the treated rats thus paralleled their excess gains in weight, as shown in table 1. The treated rats also had less nitrogen and less ash in their feces than had the controls. Both the weights of the dry feces and the percentage content of nitrogen and ash were slightly less. Whether the differences indicate better digestibility of the food or decreases in the 'metabolic' nitrogen and ash of the feces was not determined.

As compared with the eight animals in table 6, the whole group of twelve controls retained 4.3 per cent of the nitrogen, and 3.6 per cent of the ash consumed in their food, according to the carcass analyses. The twelve treated rats retained in their tissues 10.5 per cent of the nitrogen and 7.3 per cent of the ash of their food.

Energy balance. The twelve treated rats retained 6.9 per cent and the twelve controls 7.7 per cent of their total energy intake of 41,019 Calories. Thus, although the empty carcasses of the treated rats weighed 501 gm. more than those of the controls, their total energy content was 400 Calories, or 0.5 per cent, less. This was of course due to the greater fat content of the controls. The difference in percentage retention is not significant statistically because of the large individual variations in energy content. The metabolizable portion of the energy intake for the controls amounted to 34,412 Calories, or 83.9 per cent, and for the treated 34,791 Calories, or 84.8 per cent. The metabolizable portions were determined in the eight pairs used for urine and feces collection by sub-

tracting the non-utilizable energy of the feces, urine, shed hair and alimentary tract contents. All of these items were determined in the oxycalorimeter except the calorific value of the urine, which was assumed to be 8.09 Calories per gram of nitrogen.

The summated surface area for the controls for the 763 days of the experiment was 25.34 sq.m. and for the treated rats 27.17 sq.m. The average basal metabolic rates for normal rats of the same stock in over 100 determinations are 760 Cal. per day per square meter for females and 810 Cal. for males. The basal metabolic requirement for the controls for the whole period was then 19,680 Calories, or 57 per cent, of the metabolizable energy intake. They stored 3,161 Calories, or 9 per cent. Thus 66 per cent of the total metabolizable energy is accounted for, leaving 34 per cent for muscular activity and specific dynamic action. Similar figures can be approximated with somewhat less assurance for the treated rats. On the assumption that their average basal metabolic rates were the same as the controls, a total of 69 per cent of the metabolizable energy intake could be accounted for by the basal metabolic requirements and the energy stored in the carcasses. The energy used for muscular exercise and for specific dynamic action would then have been slightly less than in the controls. We have never found increases in the basal metabolic rate in rats as a result of treatment with the growth hormone, but on the contrary, have found variable decreases in the heat production per square meter of body surface (Lee and Gagnon, '30). These decreases ranged from 0 to 39 per cent and averaged about 16 per cent. If this average decrease is assumed to have occurred in the treated rats, then the portion of their total utilizable energy intake accounted for in basal metabolic requirements and in retention by the tissues was 59 per cent, leaving 41 per cent for muscular activity and specific dynamic action. The impression which was had of the somewhat greater activity of the treated animals would support these assumptions, but further objective evidence under similar conditions is necessary.

The energy cost per gram of weight gained showed striking differences in the two groups. For each gram of empty carcass weight gained the twelve controls expended 43.7 Calories, the twelve treated 26.3 Calories and the two treated, ad libitum fed rats 23.1 Calories. For each gram of gain in protein ($N \times 6.25$) the controls used 325 Calories, the treated 135 Calories and the ad libitum fed, 121 Calories.

It is of incidental interest to record the total heat production of the rats of this series under the environmental conditions which obtained, i.e., individual confinement in small cages without nest material and at a temperature of 25° to 28°C. This can be done with considerable accuracy since both the metabolizable portion of the food energy consumed and the heat value of the materials retained in the carcasses are known. The heat production per day and per square meter of body surface was: control females, 1208 Calories; control males, 1285 Calories; treated females, 1134 Calories; treated males, 1273 Calories.

Ad libitum feeders. The two treated rats given food ad libitum showed qualitatively the same responses to treatment as did their treated mates of pairs 2 and 3 on limited food intake. Their combined empty carcass weight at the end of the experiment was 527 gm., and its composition was: water, 61.5 per cent; ether extract, 12.8 per cent; fat-free dry tissue, 25.7 per cent; nitrogen, 3.3 per cent; ash, 4.4 per cent, and energy 2.4 Calories per gram. The composition of the gains is given in table 8. Their excess gains were remarkably larger in amount than those of the treated mates on paired-feeding when it is considered that their total food consumption was only 13 per cent greater. They gained 66 per cent of their initial live weight, their two control mates gained 23 per cent, and their two treated mates on paired-feeding gained 47 per cent. Likewise, the percentage retentions in the tissues of the nitrogen and ash of the food consumed showed marked differences. For nitrogen, the values were: 3.0 per cent for the controls, 7.9 per cent for the two treated on paired-feeding and 11.4 per cent for the two treated fed ad libitum. For ash the values were 2.3, 4.5 and 8.1 per cent, respectively.

The findings of Bierring and Nielson ('32) on the composition of growth hormone-treated rats under ad libitum feeding are in accord with our data on these two comparable animals.

TABLE 8

Composition of gains in two control, two treated and two treated ad libitum fed rats

GROUP	EMPTY CARCASS WEIGHT	COMPOSITION OF EMPTY CARCASS GAIN					ENERGY, CAL
		Water	Ether ext.	Fat-free dry tissue	Nitrogen	Ash	
2 controls							
Gain, grams	76.4	24.4	44.8	7.1	1.5	2.1	457
Per cent of initial datum	24.1	12.2	152.4	8.2	14.0	13.3	69
2 treated							
Gain, grams	154.0	70.3	56.2	27.5	4.0	4.0	655
Per cent of initial datum	47.7	34.4	188.0	31.2	36.3	25.5	97
2 treated, food ad lib.							
Gain, grams	215.5	126.6	38.8	50.0	6.6	8.1	593
Per cent of initial datum	69.0	63.9	134.3	58.6	61.7	53.1	91

DISCUSSION

The fact that on the same food intake the treated rats were able to show a considerable excess gain in weight, and the fact that the gain differed markedly in composition from the gain made by their control mates are strong evidence that metabolic processes were affected by the hormone. The data also give clear evidence that the growth produced has the same characteristics as the growth exhibited by young animals after puberty and before reaching their growth plateaus. Among these characteristics are the marked retention of nitrogen, the synthesis and deposition of protein and the relatively high content of water and low content of fat. The hormone apparently causes the retention of, or the return to, those metabolic characteristics which are possessed by young animals rapidly gaining in weight.

To define more precisely the specific effect of the hormone in bringing about these metabolic changes is difficult from the data of a study of this sort. All of the variables determined, except possibly fat, are closely interrelated. This is particularly shown by the constancy of the ratios of water to nitrogen or ash in all three groups of animals and in the gains of the treated and control animals. Consequently, it is not to be expected that a sharp separation of specific effects can be made by a statistical analysis of the data, although such analysis is valuable for confirmation or denial of the inferences from other considerations. The values of *t* in table 6, for example, are strictly only statistical constants from which may be derived the probabilities of the significance of the differences between the two groups. The order of magnitude of the *t* values, however, supports the inference that the constituents most specifically influenced by the hormone were nitrogen and fat-ash-free dry tissue. The increases in water and ash in the treated animals might well have been secondary effects of the increase in protein. The increased hydration observed could have occurred independently of any change in protein or fat content, but it is inconceivable that an excess gain in nitrogenous constituents (outside of hair and epidermal structures), amounting to 22.5 gm. of nitrogen could have occurred without the simultaneous retention of a considerable amount of water. A portion of the excess gain in ash can similarly be ascribed to the requirements of the added tissues, and another portion to deposition as bone.

The treated animals necessarily obtained a smaller proportion of their energy requirements from the oxidation of nitrogenous food constituents than did their controls, since they retained about 140 gm. more of protein ($N \times 6.25$). They must also have oxidized more fat than the controls, since they gained about 120 gm. less and since the total fat gain for either group was less than the fat intake. Either a specific effect of the hormone on the oxidation, synthesis or storage of fat, or the necessity of obtaining more of their energy expenditure from fat combustion, may account for the lower

fat content of the treated animals. Gaebler ('33) has reported that in dogs administration of the growth hormone causes a lowering of the R. Q., indicating an increased oxidation of fat.

It is apparent from the distributions shown in table 1 that the excess gain of the treated rats was greatest for the first week and declined progressively thereafter. This would be expected if there was induced in the animals a more rapid growth rate which was still limited by the same general factors conditioning the preexisting rate. There is no reason for assuming that the weight increase at first was composed chiefly of water or was not true growth. The excess nitrogen retention of the treated over the control group, as judged by urinary excretion, was greater for the first than for any subsequent week. The excess weight gain for the first week could thus all be accounted for as true body growth with even a lower ratio of water to nitrogen than that of the carcass itself.

The decline in the rate of excess gain might be considered to have been due to an increasing partial inanition if there had been also a progressive decline in the proportion of the food energy available for growth, after meeting the requirements for maintenance, specific dynamic action and muscular activity. The surface area, summated by weeks for the whole period, was only 8 per cent greater for the treated than for the controls. If the basal metabolic rates were not increased, the corresponding change in maintenance requirements is not of sufficient magnitude to be an important factor. Specific dynamic action is apparently slight in the rat (Mitchell and Carman, '26). The requirement for muscular energy, both because of an increase in the activity and the greater weight of the animals, was apparently somewhat increased.

It is doubtful, however, that the available energy alone could have been the only or even the most important limiting factor for the decrease in the rate of excess gain. The total amounts of food consumed by each group, serially for the first 8 weeks, were: 874, 1110, 1087, 1146, 1143, 1099, 1045 and 1065

gm. Thus, for the first week in which the treated rats made their greatest excess gain, the food consumption was 16 to 24 per cent less than for any subsequent week. The response of the two treated rats on ad libitum feeding offers further evidence. Their excess gain in weight over their two controls was 79 per cent greater than that of their two treated mates on paired-feeding, with a food consumption only 13 per cent greater. They also showed the same progressive decrease in the rate of excess gain from week to week. Their excess nitrogen retention, from urine analysis, also paralleled their excess gain in weight.

The impermanence of the gains in weight when administration of growth extracts is discontinued should be mentioned. After the end of a long period of such treatment there is usually a loss within a week or so of upward of one-fourth of the excess gains, and the growth curve thereafter remains fairly level for some time. After short periods of treatment the loss of the excess gains may be much greater, especially in hypophysectomized rats. For several years we have used for assay purposes 3- or 4-day periods of treatment in groups of about fifteen animals. The average losses in weight 96 hours after the last of three daily injections of potent extracts were calculated for eighty-eight such assays using over 200 rats and varying effective dosages. The loss for hypophysectomized rats amounted to 67 per cent and for normal adult females to 50 per cent of the weight gained in the preceding 72 hours.

A year previous to making the experiments which this paper describes in detail, a similar paired-feeding test was made with nine pairs of rats. It had to be ended after the third week because the supply of potent purified extract became exhausted and we were unable to prepare again a suitable extract with which to continue. The results for the short time were as striking as those from the present more complete experiment. At the end of the third week the treated rats had gained 248 gm. more than their litter-mate controls and had excreted 5.7 gm. less of nitrogen in the urine. From

the third to the fifth weeks (without extract) the treated group lost 50 gm. in weight and excreted 4.5 gm. of nitrogen more than their controls. Their weight then remained fairly constant until the ninth and tenth week when they began to show definite gains again. At the end of the tenth week, still under paired-feeding the controls had just caught up with them in weight, and had retained 1.8 gm. more of nitrogen.

SUMMARY

The effect of the growth hormone of the anterior pituitary upon the gain in weight and upon the composition of the gain was determined in rats under a paired-feeding regimen. Twelve pairs, each composed of litter-mates of the same sex, initial body weight and length were used. The food consumption in each pair was kept the same for control and treated members. After 8 to 11 weeks the animals were killed and their carcasses analyzed. Their compositions at the beginning of paired-feeding and treatment were estimated from the analyses of other litter-mates killed at that time.

The twelve controls showed the characteristic changes in body composition with age that would be expected. These changes were statistically significant decreases in the proportion of water, nitrogen, fat-free dry tissue, and ash, and increases in the percentage of fat and in the heat value of the tissue. They gained a total of 764 gm. in body weight and 18.2 cm. in length.

The twelve treated animals retained almost exactly their initial composition in all constituents and in the heat value of their tissue. Their excess gains over their control mates, on the same food intake, were: live weight, 531 gm; body length, 15 cm.; water, 447 gm.; fat-free dry tissue, 173 gm.; nitrogen, 22.5 gm.; ash, 22.4 gm.; fat, - 119 gm.; energy, - 341 calories. The composition of their gains thus differed markedly from that of the controls, but was closely similar to the initial composition of the animals themselves.

The nitrogen and ash balances determined indirectly by analyses of food, urine and feces corroborate the direct find-

ings from carcass analysis. The weekly excess nitrogen retention in the treated rats paralleled closely the weekly excess gain in weight, and indicates that even the excess gain in weight for the first week constituted true growth.

The energy expenditure was 1.7 times as great for the controls as for the treated animals per gram of weight gained, and 2.4 times as great per gram of protein gained. The treated animals burned more fat and less protein than did the controls.

Two treated animals on ad libitum feeding gave responses the same qualitatively, but greater in amount than did their treated mates on paired-feeding.

Statistical analysis of the data indicates that nitrogen and fat-ash-free dry tissues were the constituents most specifically influenced by the hormone.

We are indebted to Dr. T. M. Carpenter and to Dr. F. G. Benedict for their generous criticism, and to Mr. E. M. Jellinek for assistance in the statistical treatment of the data.

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EDITORIAL

TRANSFER OF THE JOURNAL OF NUTRITION TO THE WISTAR INSTITUTE

The first suggestion of a journal in America, devoted entirely to the subject of nutrition, came from Mr. Charles C. Thomas to the writer in the fall of 1918. After consulting several of his colleagues, the writer was obliged to report that the time did not appear to be opportune for such a venture. Nearly 10 years later, after Mr. Thomas had inaugurated a publishing business of his own, the proposal was renewed. This time consultation with several colleagues brought a different response, and in a few weeks a nucleus of five members of an editorial board was assembled and it was decided to proceed with plans for such a publication, Mr. Thomas to be publisher, and other persons, upon whom the existing group could agree unanimously, were to be added to the board until a number of approximately a dozen was reached.

The ownership of The Journal of Nutrition from the first was vested in the editorial board, later incorporated under the education law of the State of New York as the American Institute of Nutrition. The instrument of agreement with Mr. Thomas recited definite conditions under which either party might annul the contract by fulfillment of specific terms. No thought was entertained, however, by either party at the time of signing the contract (April, 1928) that the agreement was not to continue in force indefinitely. Relations between the board and publisher proceeded on a perfectly harmonious basis. It was hoped that manuscript support and the number of subscribers would increase sufficiently so that within a couple of years the journal could be published

once a month. The former outran all expectations, but the latter, on account of the economic depression, did not keep pace. The only solution of these difficulties seemed to be to seek a publisher who was not dependent solely upon income from the journal to defray the costs of printing and publishing. The board found such a publisher in The Wistar Institute of Anatomy and Biology, which for nearly 30 years has been supporting the publication of scientific journals and monographs. Negotiations with The Wistar Institute were begun in the spring of 1932 and were continued until terms satisfactory to the three parties in interest, Mr. Thomas, The Wistar Institute and the Board of Editors (who were as yet the only members of the American Institute of Nutrition), were reached, in April, 1933. This agreement called for the transfer of ownership of the journal and its assets at the beginning of 1934 to The Wistar Institute. This transfer was completed formally under date of January 3, 1934.

Ownership is now vested in The Wistar Institute, and the journal will sustain to the American Institute of Nutrition no other relationship than that of official organ.

It is fitting that the Editorial Board and The Wistar Institute should join at this time in an expression of appreciation to Mr. Charles C. Thomas, for his exceptional courtesy and fair attitude in all the recent negotiations and transactions connected with the transfer of the journal. The Managing Editor wishes further to go on record in commendation of the courteous and consistent attention given by Mr. Thomas to the interests of the journal throughout the 5-year period of his association with it, as publisher. Mr. Thomas relinquished his interest in the journal with regret, and yet without hesitancy, when he became convinced that the journal stood a better chance of fulfillment of its aims and purposes in the hands of The Wistar Institute of Anatomy and Biology.

JOHN R. MURLIN.

THE EFFECT OF HEAT UPON THE BIOLOGICAL VALUE OF MEAT PROTEIN

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Work previously reported from this laboratory (Morgan, '31) showed that cereal proteins and casein subjected to dry heat or toasting were not so well utilized for growth and did not allow such favorable nitrogen balances as did the raw cereal and casein. Since the digestibility of the toasted proteins was but little different from that of the raw, the difference in the value of the two could not be attributed to this source and so must have been due to the detrimental action of the heat on metabolic availability.

Ingvaldsen ('29), Daniel and McCollum ('29), Maynard and Tunison ('32), Schneider ('32) and Maynard, Bender and McCay ('32) have all conducted experiments on fish meals which were dried in various ways and have found that a high temperature used in the drying process was more detrimental to the nutritive value of the fish protein than a lower one. In a recent report, Fixsen and Jackson ('32) have confirmed the finding that casein suffers a marked decline in biological value as the result of prolonged heating even at 112°C.

Since meat is such an important item of food in our present diets, it was thought that a similar study of the effect of various methods of preparation on the nutritive value of meat would be profitable. A large number of figures are available giving the protein value of different meats and edible animal organs, but little is at hand concerning the effect of cooking on these figures. When work was begun on

this experiment, there were available the results of the work of Jarussowa ('29) and the first work by Scheunert and Bischoff ('30). The latter workers performed growth and reproduction experiments to determine the nutritive value of a pure meat diet prepared from raw, boiled or autoclaved meat with no addition other than minerals and vitamins, and concluded that there were no significant differences among the three different groups. These investigators realized that this work was open to criticism because the high protein which they fed might compensate to some extent for deficiencies and make it impossible to detect injuries which may have been caused by the heating. Therefore, Scheunert and Venus ('32) repeated the work, using both fresh and frozen meat, raw, boiled and fried, at a 10 per cent level in the diet. The results of this experiment, which were published after the present study was planned, again showed no significant differences among any of the meats used. Scheunert and Venus, however, did not use autoclaved meat.

The work of Jarussowa ('29) consisted in feeding diets containing ground meat which had been left raw or treated in one of the following ways: 1) boiled in water from 20 to 25 minutes; 2) boiled in a tightly covered casserole in a special salt bath for 4 hours; 3) kept in a thermos for 5 hours at a temperature from 75° at the beginning to 66° at the end. The results showed no difference in biological value. Since Jarussowa mixed 100 gm. of meat, treated in one of the ways given above, with 40 gm. of potatoes, 20 gm. of cabbage and 10 gm. of carrots, a mixed rather than a meat protein was studied.

The meat used in this experiment was bottom round of beef which was freed from connective tissue and fat and cut into 2-inch cubes. These were divided equally into four portions which were prepared in different ways. The first portion was ground raw three times and then spread out into very thin layers to dry. The second portion of meat cubes was boiled in single layers in water until the internal temperature was 84°C. This temperature was selected because previous work in this laboratory had shown that meat is thoroughly

cooked when this point is reached. The third portion of the meat was cooked in single layers in an autoclave at 15 pounds' pressure for 7 minutes. This gave the same internal temperature of 84°C. which was used in the case of the boiled meat. The last portion was autoclaved at 15 pounds' pressure for 1 hour. All the meat portions were ground to a fine powder when thoroughly dried. The water extract or broth from the cooked preparations was saved and dried along with the meat from which it was obtained.

TABLE 1

Daily endogenous nitrogen metabolism of adult male rats (on low egg diet)

NUMBER OF RATS	NUMBER OF PERIODS	AVERAGE BODY WEIGHT	N INTAKE	URINARY N	FECAL N	BODY N IN URINE PER 100 GM WEIGHT	BODY N IN FECES PER GRAM FOOD EATEN
		<i>gm</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg</i>	<i>mg.</i>
1	4	262	96	42	25	16	1.7
2	4	264	95	47	33	18	2.2
3	4	312	113	58	33	18	1.7
4	4	296	98	54	26	19	1.7
5	4	284	98	48	26	18	1.7
6	4	310	110	57	30	18	1.7

Two types of tests were made with these four portions of meat, that is, growth and balance experiments. The diets were made up as follows:

	<i>Per cent</i>
Dried beef	8.5 (1.07 to 1.16 per cent N)
Agar	4.0
Salt mixture	4.0 (Osborne & Mendel)
Hydrogenated vegetable fat ¹	10.0
Cornstarch	73.5

¹ Crisco.

Two drops of cod liver oil and 75 mg. of a yeast extract (containing 5 mg. N) were given separately daily to each animal to supply the necessary vitamins.

Rats were placed on these diets at 28 days of age and their food intakes and body weight increases noted for the 6 weeks following. The results of these observations are shown in table 3.

Six adult male rats were used for the balance experiments which were carried out in the manner previously reported (Morgan, '31). Four 5-day periods on the same diet with dried whole egg substituted for the meat were included among these balances, two periods at the beginning and two near the end of the tests. The nitrogen excretion in these periods was looked upon as representing the endogenous level, following the reasoning of Mitchell and Carman ('26). Data on these periods are shown in table 1. Two balances were obtained for each rat on each of the four meat diets and biological values calculated from the results as shown in table 2.

DISCUSSION

It is clear that there is a decrease in nutritive value of the beef protein caused by all three heating processes used. This is greatest in the meat autoclaved for 1 hour at 15 pounds' pressure and about the same in the other two cooked samples. The latter samples, boiled and autoclaved 7 minutes at 15 pounds' pressure, were cooked to the same internal temperature, 85°C., but this was probably exceeded in the sample autoclaved for 1 hour. The biological values (table 2) obtained by the balance sheet method on adult rats for the raw and autoclaved 1 hour samples differ by 11 ± 2.2 , while the raw and boiled differ by 7 ± 1.8 and the raw and autoclaved 7 minutes by 5 ± 2.1 . The significance of the first mentioned difference is obvious. The injury to the protein appears to be gradual and progressive with more prolonged and severe heating.

By the criterion of growth promoted in young rats (table 3) quite parallel differences are deduced. The raw meat is superior to all the cooked products, since each gram of raw meat protein eaten produced 0.78 ± 0.07 gm. greater gain than did that autoclaved 1 hour, 0.17 ± 0.06 more than the boiled and 0.14 ± 0.06 more than that autoclaved 7 minutes. The differences are in the same order and significant in nearly the same degree as are those shown by the balance experiments. Since the protein intake of all four groups was nearly

TABLE 2

Daily metabolic data showing biological value of beef, raw and cooked in three different ways

PREPARATION OF MEAT	EAT NUMBER	BODY WEIGHT	N INTAKE	URINARY N	FECAL N	ENDOGENOUS N		FOOD N ABSORBED	FOOD N RETAINED	BIOLOGICAL VALUE	AVERAGE
						Urine	Feces				
		gm.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	per cent	per cent
Raw	1	252	152	103	25	44	23	150	91	61	67±1.5
		253	163	109	29	44	24	158	93	59	
	2	260	169	100	36	54	35	168	122	72	
		262	184	105	37	55	38	184	134	73	
	3	275	157	94	27	54	23	153	113	73	
		274	140	92	25	54	21	136	98	72	
	4	271	153	109	27	53	23	149	93	62	
		317	176	122	35	55	26	167	100	60	
	5	265	124	92	22	56	19	121	85	70	
		263	155	103	22	55	23	155	107	69	
	6	304	189	121	28	68	27	188	125	66	
		356	208	127	48	63	33	193	129	67	
Boiled at ordinary pressure	1	252	134	102	23	44	21	132	74	56	60±0.9
		249	141	107	27	43	23	137	73	53	
	2	246	159	109	35	52	34	158	101	64	
		248	170	115	41	52	36	165	102	62	
	3	278	127	106	23	55	20	124	73	59	
		276	150	109	29	54	24	145	90	62	
	4	272	162	116	27	53	26	161	98	61	
		272	178	127	29	53	28	177	103	58	
	5	267	158	122	26	56	25	157	91	58	
		348	209	131	32	48	30	207	124	59	
	6	289	166	112	25	55	25	166	109	65	
		294	187	123	31	56	28	184	117	63	
Autoclaved 7 minutes at 15 pounds pressure	1	290	158	119	27	42	23	154	77	50	62±1.4
		256	157	113	29	44	23	153	85	55	
	2	316	161	110	31	49	30	160	90	62	
		268	159	110	35	56	35	159	104	65	
	3	362	138	106	25	59	21	134	87	65	
		274	206	123	33	54	32	205	136	66	
	4	319	196	124	31	55	30	195	126	65	
		273	165	112	26	53	26	665	106	64	
	5	270	161	108	25	57	25	161	110	68	
		272	139	107	21	57	22	139	89	64	
	6	346	189	135	30	61	30	189	115	61	
		313	177	127	31	59	26	174	105	60	
Autoclaved 1 hour at 15 pounds pressure	1	257	151	121	26	45	24	149	73	49	56±1.6
		282	140	112	24	41	21	137	66	48	
	2	302	161	104	34	47	32	159	102	64	
		277	173	119	41	58	38	170	109	64	
	3	372	128	109	27	61	21	122	74	60	
		380	131	112	26	62	21	126	76	60	
	4	286	191	142	36	56	30	185	99	53	
		290	190	141	32	56	30	188	103	54	
	5	270	153	132	23	57	24	153	78	51	
		272	163	132	25	57	26	163	88	54	
	6	328	156	121	26	58	27	156	93	61	
		315	193	136	32	60	29	190	114	59	

the same, the growth differences observed may be fairly ascribed to variations in the nutritive value of the meat protein preparations. The actual total food intake of the group on raw meat diet was somewhat larger than in the other three groups but since the protein content of this diet was slightly lower than that of the others the nitrogen intake remained the same as in the other groups. There may, nevertheless, be some advantage gained for the raw meat diet through the larger average caloric intake. Both total and protein intakes, however, were nearly identical in the three groups fed the cooked meat diets but growth response in the animals given the meat autoclaved 1 hour was significantly

TABLE 3

Growth of rats during 6 weeks on diets containing 8.5 per cent dried beef (7 per cent protein) raw and cooked in three different ways

PREPARATION OF MEAT	PROTEIN CONTENT OF DIET	NUMBER OF RATS	AVERAGE BODY WEIGHTS			TOTAL PROTEIN INTAKE	TOTAL FOOD EATEN	GAIN IN BODY WEIGHT PER GRAM PROTEIN EATEN
			Initial	Final	Gain			
	<i>per cent</i>		<i>gm</i>	<i>gm.</i>	<i>gm.</i>	<i>gm</i>	<i>gm.</i>	<i>gm.</i>
Raw	6.7	17	58	126	68	26.4	394	2.58±0.04
Boiled	7.1	18	58	117	59	24.5	345	2.41±0.06
Autoclaved 7 minutes	7.1	18	57	118	61	25.0	352	2.44±0.06
Autoclaved 1 hour	7.3	18	58	104	46	26.0	356	1.80±0.05

lower than in the others. All of the conditions required for definite establishment of inferiority of the latter diet appear to be fulfilled in the three cooked meat diets at least.

The digestibility data shown in table 2 indicate that the decrease in value of the cooked meat proteins is not due to a lowering in absorption since the digestibility coefficient of the raw beef protein is 97 and that of the cooked samples, 98, 99 and 98. The same observation was made in experiments upon heated gluten and casein previously reported (Morgan, '31).

An interesting difference between the biological value of meat proteins for maintenance and for growth when fed at this low level, 7 per cent, is seen in these data. The growth method of Osborne, Mendel and Ferry ('16) indicates a high

classification for beef nitrogen. Casein fed at this level, 7 per cent, produces almost no growth, while the raw beef produced nearly normal growth. Cereal proteins were found by Osborne and Mendel ('20) to afford gains of 1.4 to 1.8 gm. per gram of protein eaten at 5 to 10 per cent levels for 4-week periods, and casein 1.45 at 9.3 per cent level (Osborne and Mendel, '16). Wheat gluten was found by Morgan ('31) to allow growth for 8 weeks at the rate of 1.3 gm. per gram of protein eaten at 7 to 15 per cent levels. Hoagland and Snider ('26 a), using a 10 per cent level and 60-day period, report 2.37 for beef, 2.24 for pork, 2.21 for mutton and quite similar figures for the corresponding hearts, livers and kidneys. The experiments here reported show a maximum of 2.58 at 7 per cent level for raw beef round. Milk proteins at 10 per cent level were found by Hoagland and Snider ('26) to allow 2.36 gm. gain per gram of protein eaten in 60 days. Wheat, oatmeal and beans, under similar conditions, yielded 1.58, 1.89 and 1.32 gm. gain per gram of protein eaten.

The objection may be raised that the total amounts of food and of protein eaten may be widely different on these different diets and that the amounts of protein available for growth may be affected thereby. Some of these data have been brought together in table 4 in order to discover whether this objection is valid. It will be noted that no consistent differences can be clearly ascribed to the rather small variations in intake. Thus a whole wheat diet eaten at the rate of 10.9 and 10.1 gm. per day allows growth of 1.2 and 1.68 gm. per gram of protein, whereas with intake of 7.4 gm. per day the figure is 1.58 gm. per gram of protein eaten. The conditions used for the two latter tests, however, are closely comparable with each other but not with the first experiment. Certainly both total and protein intakes recorded for the cereals by Osborne and Mendel ('20) are comparable with those of Hoagland and Snider ('26, '26 a) and with those here reported for meat and yet the growth rates recorded are significantly different for the two groups of proteins.

The biological values obtained by nitrogen balance studies obtained usually upon adult rats for the same proteins diverge from these figures. Corn and oat proteins at 5 per cent level have biological values of 72 and 79 (Mitchell, '24), wheat gluten 83, (Morgan, '31), wheat 72 (Mattill and Clayton, '30), casein 71 and 66 (Mitchell, '24; Morgan, '31). Beef round protein at 8 to 10 per cent level has a biological value of 69 (Mitchell and Carman, '26) and at 7 per cent level, according to the data here reported, of 67. All of these values lie in the same plane in contrast with the considerably larger growth figures quoted above for the meat proteins as compared with

TABLE 4
Comparison of growth values of certain proteins

PROTEIN	LEVEL	AVERAGE DAILY INTAKE		GROWTH PER GRAM PROTEIN EATEN	FEEDING PERIOD	AUTHORS
		Food	Protein			
	<i>per cent</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>days</i>	
Wheat	8	10.9	0.87	1.2	28	Osborne & Mendel ('20)
Rye	8	10.7	0.85	1.5	28	Osborne & Mendel ('20)
Oats	8	8.3	0.66	1.4	28	Osborne & Mendel ('20)
Barley	8	12.8	1.02	1.8	28	Osborne & Mendel ('20)
Casein	9.3	6.9	0.61	1.45	28	Osborne, Mendel & Ferry (16)
Casem	8.0	5.7	0.49	1.36	35	Morgan, A. F. (unpublished data)
Wheat gluten	7- 9	6.2	0.56	1.31	56	Morgan ('31)
Wheat gluten	10-12	6.0	0.75	1.29	56	Morgan ('31)
Wheat	10	10.1	1.01	1.68	56	Morgan ('31)
Wheat	10	7.4	0.74	1.58	60	Hoagland & Snider ('27)
Oatmeal	10	7.0	0.70	1.89	60	Hoagland & Snider ('27)
Beans	10	6.6	0.66	1.32	60	Hoagland & Snider ('27)
Milk	10	8.6	0.86	2.36	60	Hoagland & Snider ('26, '26a)
Beef	10	9.7	0.97	2.37	60	Hoagland & Snider ('26, '26a)
Pork	10	9.0	0.90	2.24	60	Hoagland & Snider ('26, '26a)
Mutton	10	9.5	0.95	2.21	60	Hoagland & Snider ('26, '26a)
Beef, raw	7	9.3	0.65	2.58	42	This report
Beef, boiled	7	8.2	0.57	2.41	42	This report
Beef, auto-claved 7 min.	7	8.3	0.58	2.44	42	This report
Beef, auto-claved 1 hour	7	8.4	0.59	1.80	42	This report

cereal proteins and casein. Such a discrepancy is not observable in the values for maintenance and growth of milk and egg proteins. Biological values for these at 8 to 10 per cent levels are 94 and 85 (Mitchell and Carman, '26). At 5 per cent level, Mattill and Clayton ('30) found 89 for milk, Mitchell ('24), 93. The growth value for milk is given by Hoagland and Snider ('26) as 2.36 gm. gain per gram of protein eaten. Comparable growth data for whole egg proteins appear not to be available.

It may be fair to state then that weight gains of 2 to 3 gm. per gram of food protein eaten are made by young rats when proteins of high biological value, such as meat, milk or eggs, are fed at 5 to 10 per cent level and that the gains are only 1 to 2 gm. per gram of food protein eaten when casein and cereal grains are fed. The corresponding biological values at 5 to 10 per cent levels for maintenance of adult rats are 93 and 85 or 89 for eggs and milk, 67 to 69 for beef and 60 to 70 for casein and cereal grains. Thus beef protein falls in the higher group for growth but the lower for maintenance. It should be noted, however, that the value 69 for beef was obtained by Mitchell and Carman ('26) in experiments upon young growing rats.

An explanation for this curious discrepancy is not obvious, since the reverse condition might ordinarily be expected. The theory of McCollum and Steenbock ('12) of a qualitative difference between tissue protein synthesis and repair may be recalled in this connection. It is possible that the chief demands for amino acids in adult maintenance are quite different from those involved in the growth of tissues. The synthesis of proteins of the digestive juices and of the internal secretions may make up the major needs in the adult and the value of meat muscle proteins for these purposes may be markedly lower than for tissue growth. The reported inferiority (Nelson, Irwin and Peet, '30) of beef muscle for the support of lactation favors this suggestion.

These findings, if confirmed, may point to the validity of the popular impression that meat is less valuable to, and less

well tolerated by, persons in middle and old age than by growing children. Further studies are needed, however, applying the criterion of analyzed body weight increases to adult and the balance method to young growing animals.

EXPERIMENT WITH HORSE MEAT

A large sample of horse meat, lean round, became available¹ soon after the beef studies were completed and was used for two series of similar growth experiments. The rats ate this diet so well that it was necessary to reduce the protein content to 5 per cent in order to obtain intakes comparable with those of the beef experiment.

TABLE 5
Growth of rats during 6 weeks on diets containing dried horse meat

PREPARATION	PROTEIN CONTENT OF DIET	NUMBER OF RATS	AVERAGE BODY WEIGHTS			TOTAL PROTEIN INTAKE	TOTAL FOOD EATEN	GAIN IN BODY WEIGHT PER GRAM OF PROTEIN EATEN
			Initial	Final	Gain			
	<i>per cent</i>		<i>gm.</i>	<i>gm.</i>	<i>gm.</i>			
Raw	5.0	15	52	127	75	25.3	507	2.96
Raw	6.7	9	48	134	86	32.1	473	2.68
Autoclaved 1 hour	5.4	15	51	112	61	24.6	456	2.48
Autoclaved 1 hour	6.8	9	48	106	58	26.2	486	2.21

The meat had been ground immediately after cutting and a portion was at once dried at low temperature to be used as the raw sample. Another portion was autoclaved for 1 hour at 15 pounds' pressure. Because of the fineness of division it was difficult to obtain uniform penetration of heat during the autoclaving even though the meat was separated by tying it up in small cheese-cloth bags.

As is shown in table 5 the total food intakes were in all cases larger than were those of the beef-fed rats although the protein intakes were nearly the same as in the beef series

¹ Through the courtesy of Dr. C. M. Haring of the Division of Veterinary Science, University of California.

except for the 6.7 per cent raw horse meat diet.² Nevertheless, the gains per gram of horse meat protein were larger than in the beef series, 2.96 and 2.48 gm. gain per gram of raw and autoclaved horse meat protein eaten on total protein intakes similar to those yielding 2.58 and 1.80, in the beef experiments. However, if level of protein fed be considered the gains on 6.8 per cent horseflesh were quite comparable with those on 6.7 per cent beef, 2.68 and 2.58. The higher value seen in the autoclaved horse meat series, 2.21 as compared with 1.80 may be due to the less efficient subdivision of the meat during autoclaving.

These results on horse meat are offered chiefly for their possible value in current discussions of dog rations.

SUMMARY

1. The biological value for maintenance of raw beef muscle protein at 7 per cent level tested on rats was found to be greater than that of the same meat cooked in three different ways. The figure for the raw beef is 67, for that boiled at ordinary pressure, 60 (to internal temperature of 85°C.), boiled for 7 minutes at 15 pounds' pressure, 62 (to internal temperature of 85°C.), boiled for 1 hour at 15 pounds' pressure, 56. There appears to occur a heat injury to the protein increasing in severity with the length of exposure and the height of the temperature reached.

2. Young rats were fed these rations for 6 weeks and were found to grow best on the raw beef diet. The gain in body weight per gram of protein eaten was 2.58 ± 0.04 on the raw beef, 2.41 ± 0.06 and 2.44 ± 0.06 on the boiled and autoclaved 7 minutes' preparations and 1.80 ± 0.05 on that autoclaved 1 hour. The food intake was slightly larger on the raw diet than on the other three but not sufficiently to account for the extra growth seen.

² The assistance of Louise Kimmel in this part of the experiment is acknowledged gratefully.

3. Horseflesh prepared raw and autoclaved for 1 hour by similar methods was fed at 5 and 6.8 per cent levels in the same basal diet to young rats. The total intakes on these diets were in all cases considerably larger than on the beef diets but at a comparable level the gain in weight of the rats in 6 weeks per gram of raw horseflesh protein eaten was similar to that for beef, 2.68. The autoclaved horseflesh yielded a higher value than the similar beef, 2.21 as compared with 1.80, due perhaps to poorer heat penetration in the former.

4. Attention is drawn to the discrepancy between the high values for growth of the beef protein, parallel with the best values obtained on other animal proteins, and the lower values for maintenance shown by the biological values, parallel with casein and the cereal proteins. This may be due to sharp differences between the mechanisms of the endogenous protein metabolism of growth and maintenance.

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THE INFLUENCE OF ROUGHAGE ON PROTEIN DIGESTIBILITY

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It is not unusual in metabolism experiments in China and India to find coefficients of protein utilization in mixed diets as low as 40 and 50 per cent. This low figure must mainly be due to the use of vegetable proteins of intrinsically low digestibility and low biological value, but the question also is raised whether it may not be due in part to the bulk of the diet. In China, materials of lesser nutritive value, such as chaff and even clay, are included in the diet during famine or times of failing food supply. A lowered degree of utilization, resulting in an appreciable loss of protein material, might be of pronounced economic importance in a country where food protein is a limiting factor in nutrition.

The classical experiments of Rubner ('18) demonstrated that the ingestion of foods which contain fibrous cellular substance tends to cause low values for protein digestibility. Whitacre, Willard and Blunt ('29) more recently report a lowering of protein digestibility in man due to fiber in the diet. Weinert ('31), however, fed to sheep a ration containing 31 per cent of quartz sand, and found no marked effect on the degree of nitrogen utilization.

The following experiments with rats and with human subjects were designed to secure data on the manner in which the degree of digestibility of food protein is affected by the inclusion of exceptionally large amounts of roughage bulk in the diet.

EXPERIMENTS AND OBSERVATIONS

Experiment 1. The effect of varying the total amount of food on the digestibility of rice in rats. Two rats were placed in separate galvanized wire cages with false bottoms. The cages rested in glazed dishes in which were spread layers of filter paper so that feces might not be contaminated with urine. Steamed rice was fed in varying amounts over three periods, the amount per day being held constant for each period of 6 days. Rice was chosen because it has a high coefficient of digestibility and because it could be prepared in a semi-pasty form, not readily scattered by the rats. Vitamins were supplied by a daily dose of 5 drops of cod liver oil and 0.5 gm. of yeast for each rat. The feces were collected over exact 72-hour intervals, this interval representing the last 3 days of each period; no marker was used. The food was cooked with the same amount of water once every day, and the intake recorded on the dry basis. In the first period each rat was given as much as it desired; this was then reduced to two-thirds and finally to one-third for the succeeding periods. The rice in the second and third periods was fed twice daily so that the rats might not eat the entire amount at once. Nitrogen determinations were made by the Kjeldahl method and the digestibility calculated from the formula:

$$\text{Digestibility} = \frac{\text{Food N} - (\text{fecal N} - \text{metabolic N})}{\text{food N}}$$

The results shown in table 1 indicate that varying the daily amount of nutrient food does not alter the degree of digestibility.

Experiment 2. The effect of varying the proportion of added bulk on the digestibility of rice in rats. This was carried out with rats in the same manner as in the preceding experiment, except that while rice was used as the food material, roughage material was added in gradually increasing proportions. Each dietary period was in most cases 6 days in length, metabolism measurements being confined to the last 3 days. Toward the end of each series when some of the animals at high roughage levels began to lose appetite, it was found expedient to decrease each period to 5 days.

Four different varieties of roughage materials were used: filter paper, agar agar, China clay, and rice chaff. The China clay, secured from Kiukiang, Kiangsi, was white in color and a good grade of kaolin. In famine years the very poor peasants eat this clay in large amounts with rice or wheat and a green vegetable; it is called by them Kwan Yin Fen (Goddess of Mercy flour), being looked upon as a gift from the goddess to the poor. The rice chaff is also a substance added to the food in times of famine in many parts of China. The alimentary tract of vegetarians becomes habituated to a large bulk, and its demand for satisfaction in terms of large volume of food is very real.

TABLE 1

Effect of varying the total amount of intake on the protein digestibility of rice in rats (for 3-day periods)

EAT NO	PERIOD	BODY WEIGHT, GM.	INTAKE		FECAL NITROGEN, MG.	DIGESTIBILITY ¹
			Rice, Gm.	Nitrogen, Mg.		
1025	1	60	31.4	482	55.4	97.7
	2	61	19.0	292	33.4	97.6
	3	53	9.6	147	16.4	98.0
1026	1	55	24.8	380	46.1	97.1
	2	56	13.3	204	26.5	96.0
	3	49	8.0	123	12.2	99.2

¹ Metabolic nitrogen is estimated at 1.4 mg. per gram of food intake; see experiment 2.

The filter paper and kaolin were nitrogen-free, the agar agar was almost nitrogen-free (0.2 per cent of nitrogen, dry basis), while the rice chaff had a nitrogen content equal to that of rice. The nitrogen of rice chaff is very poorly utilized in the alimentary tract; preliminary determinations indicated that its digestibility was 40 per cent, and allowance was made accordingly in calculating the digestibility figures for this ration. The roughage materials in each case were mixed with the rice during cooking, special care being taken to secure an intimate mixture. Non-scattering food cups were used. The food was fed in all cases ad libitum; intake and level of the bulk material are all recorded on a dry basis. Fecal

nitrogen was determined in the composite 3-day sample. A small amount of greens was given at the beginning of each period, and vitamins were supplied by feeding yeast and cod liver oil as above in uniform daily doses. The nitrogen of the yeast was neglected in the calculations.

TABLE 2

Effect of varying the level of roughage intake on the protein digestibility of rice in rats. (Averages per rat per day; 3-day periods except as noted; three rats in each group)

	TOTAL FOOD INTAKE, GM	NITRO- GEN INTAKE, MG	FECAL NITRO- GEN, MG	DIGESTI- BILITY ²	TOTAL FOOD INTAKE, GM	NITRO- GEN INTAKE, MG	FECAL NITRO- GEN, MG	DIGESTI- BILITY ²
Roughage material	Filter paper				Rice chaff ³			
<i>Per cent</i>								
0	12.0	186	37	90	18.6	286	67	86
5	10.1	147	26	92	18.4	283	65	87
10	10.1	139	29	89	22.4	344	88	88
20	9.9	122	25	91 ¹	18.3	281	57	87 ¹
0	17.7	272	56	89 ¹	23.5	360	102	81 ¹
Roughage material	Clay				Agar agar			
<i>Per cent</i>								
0	22.0	341	69	89	12.5	193	42	87
5	22.0	321	71	88	10.4	152	39	84
10	21.8	300	68	88	10.1	139	40	81
20	24.0	295	56	92 ¹	6.4	78	27	75
0	24.6	379	76	89 ¹	12.9	198	46	86
40	26.9	247	58	91 ¹	8.5	78	39	66 ¹
60	35.0	214	57	96 ¹	—	—	—	—
80	54.7	169	101	86 ¹	—	—	—	—

¹ Two-day periods.

² Metabolic nitrogen reckoned at 1.4 mg. per gram of food intake.

³ Nitrogen content = 1.53 per cent; digestibility of rice chaff reckoned at 40 per cent.

The summarized results are shown in table 2. Digestibility was calculated by the above-mentioned formula. In all cases the animals, after being carried through several stages of increasing roughage intake, were again returned to a zero level. This served to demonstrate that the basic level was definite and unchanging, and also that the effects produced

were definitely related to the addition of roughage material. It was not possible to increase the proportion of roughage material to the same degree in all four cases, inasmuch as the rats refused to consume certain of the food mixtures when the per cent of roughage was increased to the highest levels. The diets most highly relished by the rats were the clay-rice diets. Filter paper tended to cause digestive difficulties; one rat died of intestinal obstruction on a 40 per cent filter paper intake. The rats employed weighed from 75 to 150 gm.; there were three rats in each of the four groups. The growth records of the rats are not regarded as significant and are not reproduced here; growth of all animals was satisfactory except where the roughage intake reached a level of 50 per cent or over. Frey, Harding and Helmbold ('28) obtained excellent growth with rats fed diets containing 25 per cent of rice cellulose.

One of the complicating factors in the calculation of digestibility is the metabolic nitrogen. For each of the rats used in this experiment, we determined metabolic nitrogen by feeding a nitrogen-free diet of cornstarch only, with the usual amounts of vitamins. The values obtained ranged from 1.15 to 1.70 mg., with an average of 1.43 mg. of nitrogen per gram of food intake. Mitchell ('24) concludes from his experiments that the amount of fecal nitrogen of endogenous origin is proportional to the weight of dry food ingested, and we have assumed that this is true. He also notes that this metabolic nitrogen coefficient increases when filter paper is added to the diet. Our observations confirm this, but the coefficient does not seem to vary directly as the amount of roughage added. Boas Fixsen and Jackson ('32) indicate that, with adult rats at least, the actual amount of metabolic nitrogen in the feces is a constant for each individual rat irrespective of the intake. But, whatever the correct manner of representing this factor, it is apparent that with a high nitrogen intake the correction for nitrogen of endogenous origin becomes less significant. It was decided therefore to employ the single figure, 1.4 mg., to express the metabolic nitrogen per gram of food intake for

all intake levels throughout experiments 1 and 2. Introducing into the calculations a figure for metabolic nitrogen slightly higher for each increasing roughage level, it is seen, would tend to give slightly higher rather than lower values for the percentage digestibility.

In table 3 are recorded the average dry weights of feces per gram of intake for the roughage-containing diets at 0, 5, and 10 per cent levels, collected over the 3-day metabolism period. The feces were all well formed. Measurements made with these rats, using carmine markers, showed that the agar agar diets required from 5 to 7 hours to pass through the digestive tract, while the other three diets required 9 to 12

TABLE 3

Average dry weight of feces of rats fed different bulk materials, totals for 3-day periods (in grams per gram of dry food intake)

		ROUGHAGE MATERIAL			
		Filter paper	Agar agar	Clay	Rice chaff
	NUMBER OF RATS	3	3	3	3
Level of roughage intake	Per cent				
	0	0.043	0.042	0.042	0.048
	5	0.103	0.112	0.082	0.065
	10	0.146	0.169	0.133	0.092

hours. The diet of rice only, without roughage, also required 9 to 12 hours. Agar agar possessed outstanding laxative properties, not only causing the material to pass through the alimentary tract with marked rapidity but also producing softer feces. In none of the other cases did the added roughage material appear to change either the character of the feces or the frequency of defecation. From table 3 it may be noted that the increase of fecal weight with the rats fed clay and also those fed filter paper was practically equal to that of the roughage material fed. Agar agar, however, definitely caused an increase in dry fecal weight; in other words it acted as a feces former.

Experiment 3. The effect of the addition of roughage bulk material on the digestibility of a meat-rice diet in man. In this experiment, instead of feeding pure filter paper cellulose, we employed cellulose prepared from cabbage, using human subjects. Two Chinese, accustomed to the monotonous cabbage-cereal diet of north China, both 20 years of age and about 55 kilos body weight, were fed a meat-rice diet to which was added cabbage fiber in amounts approximating and even exceeding the large bulk of cabbage to which the north China peasant is accustomed. The cabbage fiber was prepared by cooking the stems of cabbage with water until very soft. The material was then strained in cloth bags, and pressed free from water. It was then cooked again, with fresh water, strained and pressed again, and finally sun-dried. The product represented 2 per cent by weight of the original cabbage; the 150 gm. of this dry fiber fed during the second period was therefore equivalent to about 7.5 kilos of fresh cabbage, or 2.5 kilos for each day—a very considerable amount. The cabbage fiber used in the third period received the same treatment except that the final drying was omitted. These two preparations contained 1.40 and 1.33 per cent of nitrogen respectively, and showed a crude fiber content of 50 per cent, all calculated on a dry basis.

The diets fed are shown in table 4; the protein was supplied by lean meat and rice. The subjects ate three meals a day; the morning meal was a light one, consisting of lotus flour gruel; the meat and rice were cooked together, and, with cabbage fiber during the experimental periods, were consumed at noon and in the evening. The common Chinese diet is simple, easily prepared, and readily adapts itself to metabolism studies. The intake for both subjects was identical, except that water and salt were taken ad libitum. The subjects ate and lived in the laboratory. Each of the three feeding periods was 6 to 7 days in length, and succeeded each other without interval. Markers were used for the feces which were collected for the last 3 days of each period; aliquots were taken for analysis of the well-mixed 3-day sample. The food materials were secured in amounts sufficient for each entire ex-

perimental period and analyzed for nitrogen; the Kjeldahl method was used for all determinations. In calculating digestibility, no correction was made for metabolic nitrogen; that is, apparent digestibility has been calculated in this experiment. Throughout the experiment the subjects were maintained on a positive nitrogen balance. The feces after the feeding of cabbage fiber were exceedingly bulky. The very liberal amount of meat fed represented a new experience for these subjects, and each of them gained 4 to 5 kilos weight during the 3 weeks under experiments.

TABLE 4

Food intake of human subjects on high roughage diet (totals for 3-day periods)

	AVERAGE, NITROGEN CONTENT, PER CENT	PERIOD 1, GM	PERIOD 2, GM.	PERIOD 3, GM
Meat (lean)	3.26	1313	1346	1355
Rice ¹	1.53	450	630	630
Lotus root flour	0.50	450	390	390
Sugar	—	135	135	135
Salt vegetable	0.73	150	150	150
Cabbage fiber (dry)	1.40	—	150	—
Cabbage fiber (moist)	1.33 ¹	—	—	60 ¹
Total nitrogen (gm.)		57.4	57.0	55.0
Total calories (calculated)		8505	8694	8729
Total crude fiber (gm.)		4	80	35
Fraction of total nitrogen furnished by:		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
meat		82.3	73.9	75.3
rice		11.8	17.0	17.6

¹ Weights recorded on dry basis.

The results are shown in table 5. The cabbage fiber as prepared was found to take up water rapidly and in the second period the subjects drank a large amount of water with their meals. There was a very pronounced sensation of distention, though in no case was there one of real discomfort. This method of ingesting dry cabbage substance followed by water is not the normal method of consuming dietary roughage, but it offered a convenient means of feeding a large bulk of material. In the third period the cabbage fiber was eaten

in moist form, as nearly as possible in the condition in which it occurs in the normal Chinese diet. The actual amount of crude fiber consumed during this last period, as indicated in the table, was somewhat less.

DISCUSSION

The results recorded above indicate that with rats the addition of filter paper, clay, and rice chaff, certainly up to 50 per cent of the diet, does not result in a significant reduction in digestibility of the protein fraction of the food. For levels above 50 per cent there is some indication of slightly lowered digestibility. It must be pointed out, however, that these

TABLE 5

*Effect of cabbage fiber on the protein digestibility of a meat-rice diet in man
(for 3-day periods)*

SUBJECT	PERIOD	NITROGEN INTAKE, GM	FECAL NITROGEN, GM	DIGESTI- BILITY ¹
A	1 (without fiber)	57.4	5.1	92.3
	2 (dry cabbage fiber)	57.0	5.7	90.0
	3 (moist cabbage fiber)	55.0	3.8	93.1
B	1 (without fiber)	57.4	4.0	94.2
	2 (dry cabbage fiber)	57.0	7.8	86.3
	3 (moist cabbage fiber)	55.0	3.8	93.1

¹ Metabolic nitrogen has been neglected in this calculation.

higher levels involve a lowered protein consumption, and probably also a greater amount of metabolic nitrogen, both of which tend toward inaccuracy in the calculation of digestibility.

When agar agar was incorporated into the diets with rats a speedier evacuation of food material from the alimentary tract was produced and this was invariably accompanied by lowered digestibility figures. Rice chaff did not produce laxative effects. This is in interesting contrast to bran which is known to possess outstanding laxative properties.

In the experiment using human subjects (tables 4 and 5), it was found that cabbage fiber added to the diet to an amount

of 12 gm. of crude fiber per day did not lower the degree of digestibility (period 3). When the level of fiber intake was placed at twice that amount (period 2), however, there was a slightly lowered value for the digestibility and also evidence of laxative action. Cabbage fiber is not so laxative as other roughage substances that often enter into the diet.

In all the experiments we have used as the sources of protein, materials whose protein digestibility is high, and we have aimed to produce just as large bulk effects as possible. In one of the experiments with human subjects the diet contained almost 30 gm. of crude fiber per day. While the weight of water-free fiber may not be a measure of the roughage effect of fiber in food, it is evident that the effective bulk of the contents of the digestive tract in these experiments was exceedingly large. Experiments hitherto reported in the literature do not conceive of amounts of bulk and roughage in the amounts in which they occur in the vegetarian diets of the Orient. The experiments of Whitacre, Willard and Blunt ('29) for example were carried out on diets which contained, according to our calculation, about 5 or 6 gm. of crude fiber per day.

The addition of clay to the food intake is not limited to stress of famine, but in some districts represents a normal habit. Kaolin, of course, is in use clinically; it acts presumably as an adsorbent for pathogenic organisms in the intestinal tract. Clay eaters are to be found in many parts of China, and individuals who indulge in this habit report that clay has a smooth, pleasant taste and produces a feeling of well-being. Of the various roughage substances employed in our experiments with rats, the clay diets alone were consumed with great eagerness by the animals; they manifested this appetite even for mixtures containing 80 per cent of the clay. The only obstacle was the difficulty of securing enough genuine food nutrient at these high levels of clay intake.

A lowered digestibility of protein in diets high in certain roughage materials would appear to have three possible explanations: 1) a bulk effect, where the large amount of ma-

terial may interfere with the action of proteolytic enzymes; 2) an exceptionally large output of metabolic nitrogen, 3) an unusually rapid passage of material along the alimentary tract, resulting in incomplete absorption.

The experiments here reported, where inert substances such as clay and pure cellulose filter paper were added to the diet, indicate that there is no bulk effect leading to reduced digestibility. On the other hand, if metabolic nitrogen, which may perhaps be regarded as an index of the amount of digestive juices secreted, increases with a bulkier food intake, it would seem plausible to suggest that the alimentary tract automatically counteracts a threatened reduction in digestibility due to bulk by causing an increased flow of digestive juice. It should also be pointed out that the roughage material may actually augment the reactive surface in the alimentary tract and thus tend to promote rather than impede digestibility.

The metabolic nitrogen is a complicating factor in all digestibility experiments. Just how much of the fecal nitrogen represents undigested food substances and how much represents unabsorbed metabolic residues is difficult to determine. The results with inert materials like clay recorded in this report show at least that these substances do not react on the intestinal mucosa in such a way as to yield excessive amounts of metabolic nitrogen.

Laxative action may be said to be characterized by softer, more watery stools, and by a more rapid passage of food through the alimentary tract. It would appear that not all bulky foods are laxative in character. Others are laxative to varying degrees; and crude fiber itself from different sources apparently differs in laxative value. Ordinary cellular food fiber is a milder laxative than agar agar. Many of the cases of lowered digestibility with fiber-rich diets, bran bread, etc., reported in the literature may be best attributed to this type of action which shortens the time allowed for digestion and absorption. Our own experience, as well as that of many others, with metabolism studies on man indicates that the degree of protein digestibility varies considerably in mixed

diets. Ferrari ('32), for example, finds that the addition of meat increases the amount of bread utilized in the diet, and concludes that nitrogen digestibility is only reduced in those cases where the passage of food material through the intestinal tract is more rapid than usual. Baumann ('32), studying intestinal action on a diet of apples, a food material especially rich in fiber, also concludes that such substances act mainly to accelerate the passage of material through the intestines.

SUMMARY

Filter paper, China clay, and rice chaff have been fed to rats at different levels with a cooked rice diet. The results indicate that neither varying the total amount of food nor adding large amounts of these bulk materials produces any significant effect upon the degree of digestibility of the food protein. China clay was fed in levels up to 80 per cent of the total intake. It would appear that great bulk in the alimentary canal does not interfere with the action of proteolytic enzymes.

Agar agar caused a rapid passage of the food material through the alimentary tract and showed distinctly lowered values for protein digestibility.

An experiment with two human subjects in which cabbage fiber was fed with a meat-rice diet showed a slight tendency toward a lowered degree of protein digestibility only when the fiber was ingested in an abnormally large amount.

It is suggested that lowered values for nitrogen digestibility on a given diet result only when the food material passes through the alimentary tract with unusual rapidity.

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THE BIOLOGICAL AVAILABILITY OF SOYBEAN CARBOHYDRATE

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While soybean protein has received much attention, the study of soybean carbohydrate has been largely neglected. Soybean contains about 40 per cent of protein and 30 per cent of carbohydrate, the latter being analyzed as 'carbohydrate by difference.' This carbohydrate fraction contains little if any starch, but consists mainly of pentosans, galactans, hemicelluloses, etc., which it has been vaguely supposed the animal body utilizes in part at least. Millions of people in the Orient consume the soybean as food, and are dependent upon it as a source of both protein and carbohydrate. There is a conspicuous lack of published data showing the degree to which soybean carbohydrate is utilized by the animal body.

Blondell (1880) reported the absence of starch in soybean. Meissl and Böcker (1883) however found that soybean does contain a small amount of starch, and Harz (1885) indicated that this varies with the degree of maturity of the plant. Levallois (1881) identified galactan in soybean; Schulze and Frankfurt (1894) proved the presence of cane sugar; and Borghesani ('07) found pentose and pentosan. The most complete analytical study of soybean carbohydrate of which we are aware is that of Street and Bailey ('15), who show that sugars, starch and dextrin constitute about one-fourth of the total carbohydrate and point out that these are presumably absorbed by the human body. The remaining three-fourths consists mainly of waxes, tannins, galactans, pentosans, and cellulose, which have been assumed to be non-available to the human body.

The time-honored method of determining digestibility by measuring food and fecal carbohydrate over a definite feeding period has been used by several investigators. Oshima ('05) reports two experiments which gave 78 per cent as the average digestibility of soybean carbohydrate. Bowers ('19), also using this method, concluded that soybean carbohydrate is 96 per cent digestible. Results by this method, it is recognized, are complicated by the influence of bacterial decomposition in the gut. Experiments with animals, carnivora as well as herbivora, have demonstrated that even filter paper disappears in the digestive tract (Rubner, '16; Thomas et al., '18), while Strauch ('13) reports that 6 to 16 per cent of cellulose was 'digested' by human subjects. This method obviously is not an accurate measure of biological value, and has not been used in this study.

For estimating the biological availability of carbohydrates it was proposed to employ the following methods: 1) determination of reducing sugars after hydrolysis with taka-diastase in vitro; 2) the formation of glycogen and determination of blood-sugar level; 3) determination of extra glucose in phlorhizinized animals; 4) determination of the respiratory quotient. The last three in vivo methods were carried out using albino rats. Methods for determining the biological availability of carbohydrates have been reviewed by Morgan, Strauch, and Blue ('30) and also by McCance and Lawrence ('29).

EXPERIMENTAL

Materials

1. *Soybean.* A quantity of yellow soybean (Peking variety), secured from a local shop, was ground to pass through a 40-mesh sieve. This material on proximate analysis showed: moisture, 6.57 per cent; ash, 4.18 per cent; protein, 39.15 per cent; fat, 21.17 per cent; carbohydrate including fiber (by difference), 28.93 per cent.

2. *Fat-free soybean.* The meal as prepared above was defatted in a Soxhlet apparatus and air dried.

3. *Fat-free soybean curd.* Soybean curd (soybean cheese), which is essentially soybean protein (glycinin), obtained from a local shop was cut into thin sheets and dried at room temperature for 3 to 4 days. It was then washed with ether to remove most of the oil, dried again, ground, and finally extracted in a Soxhlet apparatus to remove the last traces of fat. This material was used as a source of protein for the control animals. Its proximate analysis gave: protein 77.80 per cent, ash 5.26 per cent, moisture 9.26 per cent, carbohydrate (by difference) 7.68 per cent.

4. *Filter paper flour.* Ordinary filter paper was cut into strips, digested in boiling normal hydrochloric acid for 1½ hours, and set aside until a fine suspension of cellulose fiber was obtained. It was then washed until free from acid, dried on the water bath and ground to a fine powder. This material was used as the carbohydrate in the negative control diet.

Experiments and results

Experiment 1. Determination of total reducing sugars on hydrolysis with taka-diastase. The in vitro method used by Olmsted ('20) in investigating the carbohydrates of food materials was followed. Duplicate samples of 5 to 10 gm. of the fat-free soybean meal were treated with boiling water, and then with taka-diastase at 37° for 17 hours, closely adhering to the details of the method cited. The total reducing sugar was determined in an aliquot after hydrolysis with hydrochloric acid by the method of Folin-Wu. Control determinations were made on the reagents. Duplicate determinations by this method showed that carbohydrate, corresponding to 27.1 per cent of the total carbohydrate in the whole soybean is digested.

Experiment 2. Glycogen formation and blood sugar level. The general method of Cori and Cori ('26) was followed in these determinations. Rats weighing 150 to 300 gm. were used, divided into groups as shown in table 2, litter mates being distributed among the groups. They were fed the diets shown in table 1. These diets were so arranged that the

carbohydrate should represent the varying factor. Soybean curd defatted as described above proved to be a practical source of soybean protein; it contained a small amount of carbohydrate which was allowed for in calculating the equivalents of the diets in table 1; it amounted to but 3 per cent of the total carbohydrate present.

The rats raised on the stock diet were fasted for 24 hours and divided into groups; each group was then fed the pre-

TABLE 1
Composition of diets used with rats in experiments 2, 3, 4

	DIET 1 POSITIVE CONTROL	DIET 2 NEGATIVE CONTROL	DIET 3 EXPERIMENTAL
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Soybean curd (fat-free)	45 0	45.0	—
Soybean (fat-free)	—	—	70.0
Starch	23 0	—	—
Filter paper	—	23.0	—
Soybean oil	30 0	30.0	30.0
Salt mixture (Osborne and Mendel)	2.0	2.0	—
Equivalent to			
Protein	35.0	35.0	34.7
Carbohydrate	26.5	26.5	25.8
Fat	30.0	30.0	30.0
Ash	4.3	4.3	3 7
Moisture	4.2	4.2	5.8

scribed diet for 1 week to render the animals accustomed to the diet. Water was supplied ad libitum throughout all phases of the experiments. During this preliminary period of 1 week, the food intake was measured daily and the feces also preserved for subsequent examination. Following this, the rats were again fasted for 24 hours, and fed the prescribed diet for the next 24 hours. At the end of this feeding period the animals were killed by a blow on the head and decapitated. One-tenth cubic centimeter of blood was imme-

diately taken for blood sugar determination, using the Byrd micro technic ('24). The liver was at once removed, and liver glycogen was determined according to the Sahyun modification ('31) of the Pflüger method. In several cases, the body of the rat after removal of the intestinal tract was also immediately cut into pieces for determination of body glycogen by the technic of Ågren, Wilander and Jorpes ('31).

The results of this experiment are shown in table 2. This table also includes for comparison data obtained for three rats which had been fasted for 48 hours. The average figures for per cent of liver glycogen as well as those for blood sugar indicate that in nutritive value, soybean carbohydrate is to be ranked about midway between starch and cellulose.

The feces of fourteen of these rats on the three diets of this experiment were collected during the 7-day preliminary feeding period, air dried, and weighed. The animals averaged 200 gm. in weight and consumed an average of 10 gm. per day of the ration. The following figures represent the average weight of dried feces per 10 gm. of food intake.

Diet	gm.
1 (starch)	0.78
2 (cellulose)	3.04
3 (soybean carbohydrate) ...	1.49

It is evident that soybean carbohydrate cannot be regarded as an extremely high-residue food material, but falls between starch and cellulose.

Experiment 3. Urinary sugar with phlorhizinized albino rats. Olmsted ('20) fed phlorhizinized dogs a definite amount of vegetable food material and by determining the extra sugar excreted in the urine estimated the amount of carbohydrate utilized. In this experiment we have employed the diets shown in table 1. Both male and female rats were employed. They weighed from 200 to 350 gm. and had been fed on stock diet no. 5 consisting of kaoliang, millet, and soybean. During the period of phlorhizinization, they were fasted, and were kept in metabolism cages of the type devised by Levine and Smith ('25), which permitted complete collection of the urine

TABLE 2

Liver glycogen, body glycogen, and blood sugar in rats fed soybean carbohydrate

RAT NO	DIET	BODY WT	FOOD INTAKE	LIVER WT.	LIVER GLYCOGEN		BODY GLYCOGEN		BLOOD SUGAR PER 100 CC
		Gm.	Gm.	Gm.	Mg.	Per cent	Mg.	Per cent	Mg.
1248	Diet 1 (starch)	263	17	8.7	—	—	98	0.042	170
1249		263	22	8.7	—	—	92	0.042	182
1252		189	13	5.0	—	—	82	0.051	180
1171		310	11	8.6	377	4.39	78	0.030	182
1300		130	7	4.5	50	1.15	43	0.040	162
1335		190	20	5.2	103	1.98	—	—	162
1337	Positive control	196	15	5.2	125	2.40	—	—	180
1343		175	15	5.4	202	3.75	—	—	150
1164 ¹		158	9	4.8	161	3.98	—	—	151
1180 ¹		185	9	6.2	196	3.17	—	—	214
1190 ¹		239	16	9.6	137	1.43	—	—	164
					Avg.	2.78		0.041	173
1250	Diet 2 (cellulose)	173	13	5.8	—	—	60	0.042	200
1251		161	12	5.6	—	—	75	0.057	122
1234		169	11	4.7	—	—	106	0.075	151
1301		126	10	4.3	46	1.08	50	0.049	150
1172		252	13	6.9	136	1.97	96	0.040	160
1344		146	14	4.6	24	0.52	—	—	148
1339	Negative control	154	15	5.0	72	1.44	—	—	133
					Avg.	1.25		0.053	152
1163	Diet 3 (soybean carbohydrate)	213	11	6.5	147	2.23	—	—	142
1164		172	11	5.4	86	1.60	—	—	134
1181		176	10	6.4	173	2.72	—	—	192
1193		236	12	7.1	93	1.31	—	—	150
1191		267	16	—	—	—	60	0.027	170
1236		146	9	6.1	—	—	27	0.042	162
1127	Experimental diet	252	13	6.0	234	3.73	63	0.030	141
1305		135	12	5.5	69	1.26	87	0.080	176
1329		168	14	5.4	110	2.04	—	—	178
1338		183	15	5.9	113	1.90	—	—	144
					Avg.	2.10		0.045	159
1165	Fasted	214	0	5.1	11	0.21			145
1179		182	0	4.3	8	0.19			116
1192		222	0	5.4	12	0.22			100
					Avg.	0.21			120

¹ Rats 1164, 1180 and 1190 were fed diet 1 except that soybean curd (table 1) was replaced by casein.

free from feces. Water was available throughout the experiment.

Phlorhizin glycosuria was induced by a daily subcutaneous injection of 50 mg. of phlorhizin in 3 cc. of olive oil till the G/N ratio became practically constant. Urinary sugar was determined by the method of Folin and Berglund, and nitrogen by the micro method of Folin and Denis. Satisfactory G/N ratios were obtained within 2 or 3 days and the rats were then ready for the feeding tests. The G/N ratios varied between 2.55 and 3.83. Shorr, Loebel, and Richardson ('30) have shown that each rat gives its own characteristic G/N ratio. Five grams of the prescribed diet was then given to the rat until the entire food was eaten, which was usually accomplished within 12 hours. The extra sugar excreted in the urine, caused by the available carbohydrate in the diet, was calculated as the difference between total glucose and the glucose from protein. Almost half the rats when submitted to the feeding test refused to consume all the material placed in the food cup; a few developed diarrhoea and could not be used. The data reported herewith are for those animals only which had accustomed themselves to consuming the diet portion promptly when fed.

The complete data for only one of the rats are reproduced here (table 3); this table is typical of the protocols for the other animals tested. In no case was extra sugar produced on the negative control diet; it would seem that filter paper cellulose was not utilized by the rats. From the data of table 3, a utilization coefficient has been calculated, using the figures for extra glucose, by comparison with starch as 100, i.e., $3.53 \div 10.18 \times 100 = 34.7$ per cent utilized.

For six animals, the average figures obtained for the per cent of soybean carbohydrate assimilated were 34.7, 25.8, 40.4, 45.0, 37.8, 47.2, giving a mean value of 38.5 per cent.

Experiment 4. Determination of the respiratory quotient. The method of the respiratory quotient has been used by Mattill ('23) in studying the effect of vitamin B on carbohydrate utilization. We employed stock rats weighing from

150 to 300 gm. They were fasted for 24 hours, and the basal respiratory quotient determination was then made in the same room in which the animals were usually kept. A simple closed-circuit apparatus patterned after that used by Wu and Chen ('29) was employed in which a desiccator formed the respiration chamber. The carbon dioxide was absorbed in

TABLE 3

Effect of soybean carbohydrate on urinary sugar of phlorhizinized rats

Rat 1087 ♀ Body weight. before experiment, 278 gm ; after experiment, 210 gm.

DAY	FOOD INGESTED	TOTAL N IN URINE	TOTAL GLUCOSE IN URINE	G/N	GLUCOSE FROM PROTEIN (N × 3.83) ¹	CALCULATED EXTRA GLUCOSE
		Mg	Mg.			Mg
1	0	212	806	3.79	—	—
2	0	230	923	4.00	—	—
3	0	240	902	3.76	—	—
4	5 gm. diet 1 (starch)	331	2272	6.86	1268	1004
5	0	202	788	3.90	774	14
					Total	1018
6	5 gm. diet 3 (soybean carbohydrate)	219	1078	4.92	839	239
7	0	248	1064	4.25	950	114
					Total	353
8	5 gm. diet 2 (cellulose)	165	620	3.76	632	0
9	0	360	1296	3.60	1379	0
10	0	158	616	3.90	—	0
					Total	0

¹ Average G/N value for fasting rat 1087 = 3.83.

80 cc. of $\frac{1}{4}$ normal barium hydroxide, the excess of barium hydroxide being titrated with $\frac{1}{2}$ normal hydrochloric acid. The usual temperature corrections were applied in measuring the volume of oxygen and calculating the ratio CO_2/O_2 . A preliminary series of ten determinations was made with fasting animals to ascertain that the apparatus was free from leaks and that checked results were possible.

The rats used in these preliminary trials were returned to the stock diet for 1 or 2 days, and then fasted for 16 hours to remove the influence of previous food. The three experimental and control diets (table 1) were then given ad libitum for intervals of 12 hours with an interval of 16 hours' fasting between each successive diet; 6 to 10 gm. of the food were consumed in each period. At the end of each feeding period each rat was placed in the apparatus for 1 hour, and the respiratory quotient determined for four 15-minute periods. The measurement for the first 15-minute period was always discarded; the recorded figure represents the average of the other three.

TABLE 4
Respiratory quotients with rats fed soybean carbohydrate

RAT NO	FASTING	DIET 3 (SOYBEAN CARBOHYDRATE)	DIET 1 (STARCH)	DIET 2 (CELLULOSE)
1238 ♀	0.80	0.91	0.98	0.77
1238 ♀	0.82	0.88	1.01	0.83
1312 ♂	0.77	0.83	0.85	0.81
1308 ♂	0.75	0.92	0.98	0.79
Average	0.79	0.89	0.96	0.80

Results are shown in table 4. The respiratory quotient for diet 3 (soybean carbohydrate) lies between the figures obtained for diets 1 and 2, representing a biological value midway between starch and cellulose.

DISCUSSION

The four methods employed for the estimation of the biological availability of soybean carbohydrate give values varying between 27 and about 50 per cent for the fraction of total carbohydrate utilized by the animal body. Of these methods the in vitro method using taka-diastase gives the lowest value, possibly due to the fact that hemicelluloses which have nutritive value may escape hydrolysis. This method, however, still remains the most convenient rapid device for determining available carbohydrate content in food materials.

Experiments involving the determination of liver glycogen do not always lend themselves to a clear-cut interpretation of results. Fat has been shown by Gregg ('31) to yield a certain amount of glycogen; the same is true of protein in the diet. Moreover, it has been shown by Wilson and Lewis ('30) that different proteins may yield different amounts of glycogen. The arbitrary use of casein by some investigators in the control diet in glycogen experiments is therefore not above question. We have endeavored to eliminate these variables by using the same protein and the same fat in equal amounts in all the animal experiments, and in addition, both positive and negative controls were employed. It is impossible of course from the data of the glycogen experiment to assert that none of the cellulose in the negative control ration (diet 2) is converted into glycogen. Fürth and Engel ('31) report results which they interpret to indicate that cellulose forms liver glycogen. The other experiments here reported tend, however, to justify the assumption that the filter paper cellulose employed was not utilized by rats. It is of note that fasting animals give glycogen results differing widely from the animals fed a cellulose-protein-fat diet. Fasting animals cannot always serve as suitable controls. The data of table 2 also indicate that the change in the percentage figure for body glycogen is not so marked as that for liver glycogen. The experiment involving liver glycogen estimation indicated that approximately half of the soybean carbohydrate was utilized. That this method appears to yield a higher value than the taka-diastase method for the amount of soybean carbohydrate utilized is not surprising, since pentoses and hemicelluloses may also yield certain amounts of liver glycogen.

Experiments with phlorhizinized rats gave a utilization coefficient of 38 per cent, which also is somewhat higher than the figure obtained by in vitro digestion with taka-diastase. This is in agreement with the observation of Olmsted ('20), who also found higher values with phlorhizin experiments. We recovered as urinary sugar an average of 75 per cent of the starch fed to the control rats. Our results indicate that

phlorhizinized rats may be used for assaying the food value of carbohydrates.

The method of the respiratory quotient furnished an interesting check on the determination of carbohydrate utilized, but data from this type of experiment must be interpreted with caution, and this method does not commend itself as a routine method for the assay of carbohydrate in foods. The result obtained approximates that estimated from the liver glycogen method.

We have used the data of these experiments as a basis for approximating a utilization coefficient for soybean carbohydrate. The figure from taka-diastrase digestion we believe to be low, while the experiments based upon the formation of liver glycogen and upon the method of the respiratory quotient involve complex diet factors. A consideration of the data from all four methods employed suggests that of the soybean fraction usually reported as 'carbohydrate by difference' about 40 per cent is available to the animal body. We are inclined to believe that hemicelluloses ordinarily regarded as non-utilizable are, in the case of the soybean, in part available.

SUMMARY

An experimental diet containing soybean carbohydrate, with positive and negative controls, was used with rats to measure the formation of liver glycogen, extra sugar in phlorhizinized animals, and the respiratory quotient. In vitro estimation of digestibility as well as these in vivo experiments indicate that about 40 per cent of the soybean carbohydrate is utilized by the animal body.

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THE EFFECT OF THYROIDECTOMY AND THYROID FEEDING ON THE MILK SECRETION AND MILK FAT PRODUCTION OF COWS

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It has been often suggested that the initiation and cessation of the lactation cycle are regulated by hormones. While investigations have been made using organ extracts it cannot be said that the control of milk secretion has been fully explained. Fellner ('31) concludes the action of the hormones of reproductive organs in the relation to milk secretion involves two factors: one stimulating growth and development of the gland during gestation, the other inhibiting the secretion of milk by the developing gland. The removal of the placenta at parturition supposedly removes this inhibition of lactation. The investigations of Turner and his co-workers ('30, '31) fully bear out the secretion of a growth promoting substance by the corpus luteum, but the initiation of secretion is ascribed to the appearance of a stimulating agent rather than to the removal of an inhibitor. This stimulating principle has been identified by Corner ('30), Turner and Gardner ('31), and Gueter and Stricher ('29) as a product of the anterior lobe of the pituitary. Its relationship with the corpus luteum secretion has been demonstrated by Nelson and Pfiffner ('31).

While the general lactation cycle may be controlled by these hormones there is a certain amount of more or less indefinite evidence indicating that metabolic rate may be an additional factor especially in the production of milk fat.

Relatively large day-to-day variations in milk fat percentage and in milk fat production are common knowledge to all those familiar with the records of dairy cattle. It is also well known that usually the percentage of fat is lower at the morning milking than at the evening milking. McEwan and Graham ('33), Campbell ('31, '32) demonstrate this clearly. The latter author shows that there is a distinct decline in the absolute amount of fat found in morning milkings and attributes this to some factor at work at night. Bartlett ('29) has pointed out that the milk fat percentage may be affected by the stage of lactation of the animal. It tends to rise near the end of the period. Clothier ('22), Ragsdale and Turner ('22), Buchanan and Lowman ('29), and McEwen and Graham ('33) conclude that the percentage of fat in milk is highest during the winter months and lowest during the early summer. That environmental temperature is a factor influencing the milk fat percentage was shown by Hays ('26) and confirmed by Weaver and Mathews ('28). These authors show that the per cent of fat in milk is inversely proportional to the temperature. Gowan ('24) has shown that the per cent of fat in the milk of Holstein cows decreases with age after the first lactation cycle of the cow.

While it is dangerous to draw conclusions regarding the production of milk fat from milk fat percentage data, as will be pointed out below, the work cited regarding the factors influencing the daily variations in milk fat may be shown to be compatible with a theory which postulates that a relationship between milk fat production and metabolic rate exists. There appears to be an increase in milk fat under conditions in which increased metabolism may be predicted from the experimental procedure, and vice versa. The data gathered by Campbell give support to such a theory. Much of the remaining work is, however, complicated by changes in the values of milk secretion and fat percentage as neither are constant nor are the variations in the same direction.

EXPERIMENTAL

No calorimeter was available for the determination of the relationship between metabolic rate and production of milk and milk fat. Thyroidectomy and thyroid feeding were therefore used to alter the metabolic rate of animals in the experiment to be described below.

Cows from the Ontario Agricultural College herd were used. These cows were milked twice daily, at 6 A.M. and 5 P.M., throughout the experiment. The milk was weighed to the nearest 0.5 pound and sampled as described in the official method for fat determination. Analyses for fat were carried out by the Babcock method.

During the experimental period no changes were made from the ration ordinarily fed to milking cows, except when thyroid was included in the diet and when supplements were made to the food of cow no. 45, as outlined below. The animals were confined to the stable at all times.

The thyroid fed was prepared from frozen thyroid glands obtained weekly from the abattoir. This material was minced in a meat chopper and dried in acetone and ether. Final traces of ether were removed from the filter press cake with a draft of warm air, and the dried material was ground in a Wiley mill. Since fresh lots of glands were dried weekly and the amount of moisture in the final product was not constant, feeding was done on the basis of the original weight of the glands before drying.

The effects of thyroidectomy on milk and milk fat secretion were studied on three cows. The thyroid glands were removed from cow no. 45 at the end of the first week of the experiment. Similar operations, but without removing the thyroids, were performed on cows nos. 21 and 33 for control purposes. Two weeks after the control operation the thyroids were removed from cow no. 21. Thus the results of thyroidectomy may be compared not only with the normal but with data gathered from animals which underwent a precisely similar operation, except that the thyroids were left intact.

When effects of the removal of the thyroid glands had been effectively demonstrated an attempt was made to change the milk and fat secretion by feeding desiccated thyroid glands, prepared as outlined above. Various levels of this material were fed and the milk and fat production during these periods were recorded. An attempt was also made to increase the fat production of no. 45 by adding to the diet substances which might be possible precursors of milk fat. After a long normal period on two cows, nos. 5 and 6, shown to be in falling lactation, the addition of small amounts of thyroid to their diet showed clear-cut results.

TABLE 1
Cow no. 33

WEEK	MEAN DAILY MILK YIELD (LBS.)	TOTAL WEEKLY FAT YIELD (LBS.)	PER CENT OF FAT IN MILK	REMARKS
1	26	7.76	4.2	Normal Operation
2	15	4.64	4.3	
3	14	4.19	4.4	
4	10	3.45	4.9	
5	9	2.54	4.1	
6	11	3.20	4.2	
7	9	2.64	4.2	
8	8	2.18	4.0	
9	7	2.30	4.7	

The milk secretion is presented as the mean daily yield of milk during the week. The twice daily analyses have been reduced in most cases to their mean values for the week. The fat production is represented as the total amount of fat produced during the week. The fat percentage is the percentage that the total fat production for the week is of the total milk secretion for the same period.

RESULTS AND DISCUSSIONS

Milk secretion. Table 1 is a condensation of the data gathered on the milk secretion from Holstein cow no. 33. A normal period of 1 week is presented, followed by a control operation without thyroidectomy.

Table 2 presents, in condensed form, the data gathered for the milk secretion of Holstein cow no. 21 during the whole experiment. A normal period of 2 weeks is shown. The control operation was then performed and after another 2-week interval the thyroids were removed.

Table 3 similarly presents results with the Ayrshire cow no. 45. A thyroidectomy operation was performed at the end of the first week of the experiment.

From the daily results of the control operations shown in tables 8 and 9 it appears that the curve of milk secretion regains its normal slope within a week's time. The small secondary fall in milk secretion, shown in table 8, after apparent recovery, is possibly due to the fact that the incision became infected at this time and had to be drained. However, in any case recovery is apparently complete by the end of the third week after the control operation.

A comparison of the curve of milk secretion of cow no. 21 after the control operation and after thyroidectomy, shown in table 2, indicates little difference in the effect produced. This may be due to the fact that the second operation reduced the milk secretion to a very small amount. No further fall could take place without abolishing secretion entirely.

Examination of the data presented in table 3 shows that in the case of cow no. 45 there was a distinct fall in milk production for 5 weeks following thyroidectomy, followed by a flattening out of the downward slope of the curve of secretion. From tables 8 and 9 it would appear that this prolonged fall in milk secretion might possibly be attributed to thyroidectomy, and not to the simple effect of operation. However, even if thyroidectomy per se produces a diminution in the milk secretion of cows, as indicated in table 3, the results shown above are difficult to interpret because of the somewhat similar effect after the control operations.

The effect of thyroid feeding

Since the decreases in milk secretion after operations could not be definitely associated with removal of the thyroid

TABLE 2
Cow no. 21

WEEK	MEAN DAILY MILK YIELD (LBS.)	TOTAL WEEKLY FAT YIELD (LBS.)	PER CENT OF FAT IN MILK	REMARKS
1	19.0	5.55	4.2	
2	17.0	4.90	4.1	
3	12.4	3.64	4.2	Operation
4	7.7	2.17	3.9	
5	4.6	1.21	3.7	Thyroidectomy
6	3.1	0.99	4.5	
7	4.1	1.07	3.8	
8	3.8	0.95	3.5	
9	3.8	0.89	3.3	
10	3.7	0.89	3.4	
11	5.0	1.02	2.9	
12	39.8	12.53	4.5	13 lbs. thyroid fed daily: calved
13	45.6	10.67	3.3	No thyroid
14	46.9	13.92	4.2	19 lbs. thyroid fed daily
15	50.3	13.83	3.9	1.3 lbs. thyroid fed daily
16	51.4	12.99	3.6	0.66 lb. thyroid fed daily
17	50.3	14.67	4.2	0.66 lb. thyroid fed daily
18	53.3	12.68	3.4	0.66 lb. thyroid fed daily
19	54.6	12.70	3.4	0.66 lb. thyroid fed daily
20	54.6	13.09	3.4	0.66 lb. thyroid fed daily
21	44.4	10.38	3.3	0.66 lb. thyroid fed daily (no thyroid 2 days)
22	49.9	10.39	3.4	0.66 lb. thyroid fed daily
23	46.2	11.76	3.7	0.75 lb. thyroid fed daily
24	43.2	12.10	3.6	0.75 lb. thyroid fed daily
25	46.0	10.89	3.5	0.75 lb. thyroid fed daily
26	45.2	11.38	3.5	0.75 lb. thyroid fed daily
27	43.3	11.16	3.8	0.75 lb. thyroid fed daily
28	41.9	11.64	3.3	0.75 lb. thyroid fed daily
29	39.5	9.72	3.3	0.75 lb. thyroid fed daily
30	40.5	9.86	3.5	0.75 lb. thyroid fed daily
31	42.0	10.95	3.7	1.50 lbs. thyroid fed daily
32	39.0	10.19	3.7	0.50 lb. thyroid fed daily
33	39.3	9.65	3.5	0.50 lb. thyroid fed daily
34	39.4	9.06	3.4	0.50 lb. thyroid fed daily
35	33.1	7.81	3.4	No thyroid
36	25.8	5.80	3.2	No thyroid
37	25.6	5.28	2.9	No thyroid
38	25.5	5.18	2.9	No thyroid
39	25.5	5.52	3.1	No thyroid
40	29.0	6.20	3.0	0.50 lb. thyroid fed daily
41	30.3	7.78	3.7	0.50 lb. thyroid fed daily

glands, an attempt was made to study the effects of small amounts of desiccated thyroid glands in the diet of these

TABLE 3
Cow no. 45

WEEK	MEAN DAILY MILK YIELD (LBS.)	TOTAL WEEKLY FAT YIELD (LBS.)	PER CENT OF FAT IN MILK	REMARKS
1	35.0	12.44	5.1	Normal
2	34.5	11.33	4.7	Thyroidectomy
3	31.3	8.56	3.9	
4	28.0	7.57	3.9	
5	25.8	6.95	3.9	
6	23.8	6.71	4.0	
7	24.4	6.82	4.0	
8	21.8	6.66	4.4	
9	23.0	7.18	4.5	1.3 lbs. thyroid fed daily
10	28.3	9.35	4.7	1.3 lbs. thyroid fed daily
11	30.2	8.60	4.1	1.3 lbs. thyroid fed daily
12	31.5	8.72	3.9	1.3 lbs. thyroid fed daily
13	29.2	7.76	3.8	1.3 lbs. thyroid fed daily
14	27.7	7.55	3.9	1.3 lbs. thyroid fed daily
15	27.6	7.31	3.8	2.6 lbs. thyroid fed daily
16	26.4	7.24	3.9	2.6 lbs. thyroid fed daily
17	21.8	6.49	4.2	2.6 lbs. thyroid fed daily
18	22.3	5.96	3.8	2.6 lbs. thyroid fed daily
19	21.0	5.67	3.8	+ 2 lbs. sugar added daily
20	19.5	5.83	4.5	+ 2 lbs. sugar added daily
21	14.7	4.59	4.5	No sugar
22	15.5	3.85	3.5	+ 4 lbs. honey added daily
23	14.0	3.76	3.8	+ 1 lb. butterfat added daily
24	13.4	3.68	3.9	+ 1 lb. butterfat added daily
25	11.3	3.09	3.9	+ honey + fat + 1 lb. beet molasses
26	10.6	2.79	3.7	+ honey + fat + beet molasses
27	10.3	2.56	3.6	+ honey + fat + beet molasses
28	8.7	2.32	3.8	+ honey + fat + beet molasses
29	7.9	1.88	3.4	Thyroid alone
30	6.8	1.74	3.6	+ 1 lb. dried egg yolk
31	7.0	1.78	3.6	+ 2 lbs. dried egg yolk
32	4.9	1.46	4.2	No thyroid
33	1.8	0.64	5.1	No thyroid

animals. Thyroid glands, dried in the manner described above, were added to the diets of the thyroidectomized animals 21 and 45. The resulting effects are shown in tables 2 and 3.

Cow no. 21 received thyroid in the diet during the twelfth to the thirty-second weeks of the experiment. It is unfortunate that the initial effect of thyroid feeding on milk secretion is obscured by the effects of parturition. Thyroid feeding was discontinued during the thirty-fifth to the thirty-ninth weeks of the experiment. A distinct decline in milk secretion from 39.4 pounds on the preceding week to 25.8 pounds on the thirty-sixth week occurred. Resumption of thyroid feeding was followed by a rise in milk secretion. The results obtained when this cow was deprived of thyroid during the thirty-fifth to the thirty-ninth weeks simulate the effects of thyroidectomy and suggest, therefore, that the lessened milk production after thyroidectomy is indeed due to depriving the animal of its normal thyroid secretion.

The thyroidectomized cow, no. 45, was fed thyroid during the eighth to the thirty-first weeks of the experiment. As shown in table 3, this was followed by a rapid rise in milk secretion almost to the preoperative level.

These results indicate that thyroid feeding definitely increases the daily milk yield of thyroidectomized cows. If a similar effect could be shown with normal animals it would provide additional evidence that the thyroid is a factor in milk secretion. Accordingly, two Holstein cows, nos. 5 and 6, were chosen for experimental feeding. These animals were observed during a normal period of 11 weeks and were shown to be definitely in a state of falling lactation. At the end of this time an amount of thyroid equal to half a pound of the original glandular material was added to the daily ration of each animal. The consequent rise in milk secretion is shown in tables 4 and 5. Following the 4-week period thyroid feeding was discontinued and the secretion of milk rapidly fell to the levels which would have been reached in the absence of added thyroid. These results, together with those obtained with thyroidectomized animals, indicate that the thyroid gland has a definite influence on the amount of milk secreted by the cow.

THE PRODUCTION OF MILK FAT

The production of milk fat may be considered in two ways, namely, from the percentage of fat in the milk secreted and the total amount of fat produced. It is obvious that variations in the total amount of fat produced represent true variations. On the other hand, variations in the percentage of fat in the milk may be due either to variations in milk secretion or in fat production or to variation in both.

The effect of thyroidectomy and thyroid feeding will be considered in the sequence followed in discussing milk secretion.

The effect produced by a control operation on the production of milk fat is similar to that produced on milk secretion (tables 1, 2, 8, and 9). A sharp drop in production is apparent. The decrease continues for about 4 weeks, when equilibrium is reached (table 8). It is important to note, however, that after the control operation the per cent of fat in the milk of these animals is not below the limits of normal variation. Fat production and milk secretion fall at essentially the same rate.

The demonstration of the effect of thyroidectomy on the production of milk fat in cow no. 21, apart from any effect which might be due to operative disturbance without thyroid removal, is complicated by the fact that milk secretion fell almost to a minimum on the first few days after the operation. In order therefore to reproduce the effect of thyroidectomy, the dried thyroid was removed from the diet of this animal on the thirty-fifth week of the experiment. This procedure removes any complications which might be caused by an operation. The data in table 2 show that on removal of the thyroid from the diet of this animal, fat production dropped from 9.06 pounds on the thirty-fourth week to 5.80 pounds on the thirty-sixth week, or a decline in production in 2 weeks' time of 3.26 pounds of fat, or 36 per cent of the former level. The per cent of fat in the milk over the same period declined from 3.4 per cent to 2.9 per cent. A similar decline was observed after thyroidectomy operation. It ap-

pears that removal of the thyroid glands from the lactating cow causes a sharp decline in the total yield of milk fat. The diminution in the production of milk fat is more rapid than that of milk secretion. It soon reaches and is maintained approximately constant at a new and lower level.

The effect of thyroid feeding

The milk fat production of thyroidectomized cows nos. 45 and 21 fell to a new level following thyroidectomy. After this level was well established for cow no. 45 an amount of thyroid equal to 1.3 pounds of the original glands was added to the daily diet on the ninth week of the experiment. Table 3 shows that the production rose from 6.66 pounds per week on the eighth week to 9.35 pounds on the tenth week, or that there was a rise of 2.69 pounds, equal to approximately a 40 per cent increase, above that produced in the absence of thyroid influence.

In the case of cow no. 21, 0.50 pound daily of thyroid gland was fed commencing on the fortieth week of the experiment. Table 2 shows that fat production rose from 5.52 pounds on the thirty-ninth week to 7.78 pounds on the forty-first week. This rise of 2.26 pounds is approximately equal to 40 per cent of the previous production in the absence of thyroid feeding.

The distinct increase in the fat yield accompanying thyroid feeding to thyroidectomized animals suggested its trial with normal cows. The two animals already referred to, nos. 5 and 6, were chosen for this work. Table 4 shows that the fat secretion of no. 5 rose, when the equivalent of 0.50 pound of thyroid gland was fed daily, from 5.22 pounds on the eleventh week to 7.60 pounds on the fourteenth week. Table 5 shows that the cow no. 6, similarly treated, increased its fat production from 6.77 pounds to 9.48 pounds over the same period.

Clear-cut increases in both milk and milk fat production have been shown to accompany additions of thyroid substance to the diet of both thyroidectomized and normal animals. Decreases were shown after thyroidectomy and after the removal of desiccated thyroid glands from the diet. These

results might be interpreted as an indication that the thyroid gland produces a hormone which controls milk secretion, or that one of the hormones which controls the lactation cycle produces its effect through the thyroid.

Examination of the data presented in tables 2 and 3 reveals that the thyroid probably contains no such principle.

TABLE 4
Cow no. 5

WEEK	MILK	FAT SECRETION	FAT PER CENT	REMARKS
1	34.1	6.68	3.3	Normal
2	34.7	7.63	3.1	
3	34.7	7.69	3.2	
4	33.3	7.35	3.1	
5	31.8	7.10	3.2	
6	30.0	6.49	3.1	
7	28.4	5.92	3.0	
8	27.1	6.16	3.3	
9	24.4	5.62	3.3	
10	21.8	4.95	3.2	
11	22.1	5.22	3.4	
12	24.6	6.09	3.5	0.5 lb. thyroid fed daily
13	27.5	7.18	3.7	0.5 lb. thyroid fed daily
14	28.2	7.60	3.8	0.5 lb. thyroid fed daily
15	27.1	7.08	3.7	0.5 lb. thyroid fed daily
16	23.0	6.25	3.9	No thyroid fed
17	19.6	4.94	3.6	No thyroid fed
18	18.5	5.09	3.9	No thyroid fed
19	17.5	5.31	4.3	No thyroid fed
20	14.8	4.35	4.2	No thyroid fed
21	11.2	3.58	4.6	No thyroid fed
22	9.4	2.97	4.4	No thyroid fed

After the removal of the glands there is a rapid initial drop in production of milk and milk fat, followed by a general smoothing out of the curves. Neither milk nor fat production is reduced to zero. It is also clear that the secretion of milk and milk fat continued to fall in each of the periods when thyroid was fed at constant levels in the later weeks of the experiment. These periods were not designed for the purpose of deciding whether the thyroid is the predominant factor controlling lactation and objection may be justifiably

raised to the use of all the data as negative evidence. However, that shown in table 2 for cow no. 21 from the twenty-third to the thirtieth weeks of the experiment may be used for this purpose without objection. During this period milk secretion declined from 46.2 to 40.5 pounds daily and fat production from 12.10 to 9.86 pounds weekly. This diminution is slow but definite and incompatible with a theory of primary control of the lactation cycle by the thyroid gland.

TABLE 5
Cow no. 6

WEEK	MILK	FAT SECRETION	FAT PER CENT	REMARKS
1	32.0	7.63	4.0	Normal
2	32.9	9.07	3.9	
3	33.9	9.20	3.9	
4	31.1	8.53	3.9	
5	29.1	9.78	3.8	
6	30.7	8.45	3.9	
7	27.1	7.61	4.0	
8	26.6	7.16	3.8	
9	27.5	7.49	4.1	
10	26.4	7.08	3.8	
11	24.4	6.77	4.0	0.5 lb. thyroid fed daily
12	28.0	7.97	3.9	
13	34.7	9.53	3.9	
14	35.0	9.48	3.9	
15	33.6	9.00	3.8	
16	30.7	8.93	4.6	
17	25.7	6.53	3.6	
18	24.0	6.52	3.9	
19	22.9	5.96	3.7	
20	19.3	5.35	3.9	
21	18.3	4.80	3.7	
22	18.0	4.89	3.8	

The results outlined above indicate that the removal of the thyroid glands from the animal, or the feeding of desiccated thyroid in the diet, simply alters the plane of secretion of milk and milk fat, and that the thyroid does not contain any principle which controls the lactation cycle.

Since the presence or absence of the thyroid gland shows a definite effect on the secretion of milk and milk fat, which

in the light of the data at hand cannot be attributed to the secretion of a substance directly controlling the activity of the mammary gland, the effect must be due to some secondary condition produced by thyroid action. The thyroid gland of course influences the rate of metabolism in the animal. In the absence of the thyroid the basal metabolic rate falls some 30 to 40 per cent, while additions of the gland to the diet are known to increase metabolic rate in direct proportion to the amount of the active principle, thyroxin, absorbed.

If the effects shown above are due to one factor, or the sum total of the factors of increased metabolism, it is reasonable to suppose that at any time in the lactation cycle of an animal, maximal secretion of milk or milk fat can be attained, other factors being equal, only when the rate of metabolism is optimal for milk production. Above this optimal rate there would be increased catabolism. Subsequent additions of more thyroid to the diet, under these conditions, should show no increase in the milk or milk fat production, but rather a decrease, and the digestion products, which with the cow are chiefly carbohydrate and protein in origin, would be consumed in the body and the possibilities of fat formation from carbohydrate would be remote. Thus the precursors of milk fat, which according to Meigs, Blatherwick and Cary ('19), Cocke-fair ('28), Peterson, Palmer and Eckles ('29), are fatty compounds, would diminish and the secretion of milk fat would consequently be lower than the secretion of the other constituents of milk. If the metabolic rate of the animal is below the optimum for maximal milk or milk fat secretion the factors which diminish the secretion of the mammary gland will be a reduction in metabolites, coupled with a lower blood supply to the gland due to decreased heart rate. Increasing the metabolic rate under such conditions should show an increase in milk and milk fat production which cannot, however, be maintained against the decline resulting from the progression of the lactation cycle.

While a theory as outlined above cannot be definitely proved without calorimetric data, an attempt has been made to show

the behavior of two thyroidectomized cows, one kept in a state of hyper-metabolism, the other in a state of hypo-metabolism. The amounts of thyroid fed were purely arbitrary and the state of metabolism was judged from such symptoms as respiration rate, heart rate, excitability, appetite, etc.

Cow no. 45 was fed a daily quantity of thyroid, from the ninth to the fourteenth weeks, equivalent to 1.3 pounds of the original glands. The downward slope of the curve of milk secretion, after its peak, due to the initial effect of thyroid, was reached and was slightly arrested by doubling the dose of thyroid on the fifteenth week. This was followed by a marked drop in secretion from 26 to 21 pounds daily on the seventeenth week. This animal was now definitely in a state of hyper-catabolism. An addition of sugar to the diet, at the rate of 2 pounds daily, apparently delayed a further diminution of milk and fat production. This took place on the twenty-first week when sugar was removed from the diet. On the twenty-second week this downward fall in milk secretion was changed to a slight rise by the addition of 4 pounds of honey to the diet. The addition of further supplements to the ration apparently forestalled any abrupt declines in the milk secretion until thyroid feeding was discontinued. The effect of doubling the thyroid on the milk fat was a further diminution of its production. The fat percentage in the milk showed a gradual decline to the end of the experimental thyroid feeding, coincident with falling milk secretion. This indicates that increasing the thyroid fed tended to diminish the fat secretion at a more rapid rate than milk secretion. This might be ascribed to an increase in metabolism above the optimum rate, as pointed out above.

With the object of changing the milk and milk fat production at will, by altering the amount of thyroid fed, an attempt was made to keep cow no. 21 at a rate of metabolism below the optimum for secretion. As shown in table 2 no distinct difference could be observed in milk secretion when the thyroid fed was raised from 0.66 pound daily on the twenty-third week of the experiment. However, at this time fat produc-

tion rose from 10.38 pounds on the twenty-second week to 12.10 pounds on the twenty-fourth week. On the thirty-first week of the experiment the thyroid fed was increased to the equivalent of 1.50 pounds daily. Milk secretion rose slightly, showing a gain for the week of 1.5 pounds daily, and fat production figures showed a gain of a little more than 1 pound over the previous week. From the thirty-second to the thirty-fourth weeks the equivalent of 0.50 pound of thyroid was fed. Milk secretion fell off slightly, while fat production diminished from 10.19 to 7.81 pounds. The fall in both milk and fat, when thyroid was removed from the diet and the rise when it was replaced, has been discussed previously.

While these experiments show little or no information as to the actual mechanism of the changes in milk and milk fat secretion, they do show that additions of thyroid to the diet of an animal already receiving a reasonable supply of this material, will cause a decrease in both milk and milk fat production. Since sharp declines were arrested by introducing additional food of high caloric value to the diet it appears that the factor involved is the metabolic rate. An animal fed smaller amounts of thyroid may, however, have its milk and milk fat production increased by changing the amounts of thyroid in the diet. These results are at least compatible with a theory that the thyroid exerts its influence through its control of metabolic rate.

The results obtained with cow no. 19, table 6, show that some other factor than thyroid secretion controls milk fat and milk production. This animal, with a previous history of abortion, was washed, fitted for the show ring and exhibited on the fifth and sixth days of the experiment. On the tenth day an amount of thyroid was fed equal to 0.50 pound of the original glands. The animal showed a distinct decline in both milk and milk fat before thyroid was fed, indicating that the abortion was probably brought about by the treatment received in preparation for exhibition. The addition of thyroid to the diet temporarily raised the plane of secretion, as it does with animals in falling lactation. This is shown by

an arrest in the curve of the fall of milk secretion and of fat production. However, the factors governing the secretion of milk, outside the thyroid, presently forced both milk and milk fat production to lower levels, showing that the effect produced by the thyroid was secondary to other factors governing milk secretion.

TABLE 6
Cow no. 19

DAYS	MILK (LBS.)	FAT (LBS.)	REMARKS
1	37.0	1.21	
2	35.5	1.27	
3	34.5	1.19	
4	31.0	1.05	
5	34.5	1.11	Fitted
6	33.0	1.18	Exhibited
7	31.0	1.17	
8	26.0	0.96	
9	26.0	0.85	
10	26.5	0.91	0.5 lb. thyroid fed daily
11	26.5	0.85	
12	26.0	0.76	
13	25.0	0.85	
14	25.0	0.89	
15	22.0	0.71	
16	21.5	0.74	
17	21.5	0.74	
18	20.5	0.74	
19	18.5	0.71	
20	18.5	0.80	
21	18.5	0.76	
22	18.5	0.77	Aborted
23	23.0	0.92	
24	26.5	0.92	
25	31.5	1.45	

It will be noted that the effect of thyroid has been considered only on animals past the peak of milk production in their normal lactation cycle. Further evidence of the secondary nature of the effect of thyroid is afforded by a consideration of its effects soon after parturition, when the factors controlling milk secretion are probably exerting their greatest effect.

Changes were made in the amounts of thyroid fed to cow no. 21, shown in table 2, directly after parturition, when milk and fat secretion were rising rapidly. The equivalent of 1.3 pounds of thyroid glands was being fed on the twelfth week, when this animal calved. Milk secretion rose at this time from 5 pounds to 39.8 pounds daily, and fat secretion from 1.02 pounds to 12.53 pounds for the week. On the thirteenth week no thyroid was fed. Milk secretion continued to rise, showing a gain of 5.8 pounds over the previous week. Fat secretion diminished 1.86 pounds for the week. Milk secretion continued to rise in the following weeks without regard to the amount of thyroid fed, as did fat production until a peak was reached. In the succeeding weeks, that is in the normal period of falling lactation, the effect of thyroid could again be demonstrated. It is of interest to note here that during the stage of increasing milk production in spite of the apparent ineffectiveness of thyroid on the milk secretion, some slight effects on fat secretion are apparent; also, that thyroid at this time was absolutely necessary to the well-being of the animal, which showed serious symptoms when the thyroid feeding was stopped and was only saved from death by restoring thyroid to its diet. Thyroid feeding was, therefore, necessary to maintain life in this thyroidectomized animal. During the thirteenth week the temperature of this cow rose to 107° and quickly fell to normal when thyroid feeding was resumed. The diminution in fat secretion at this time might have been caused by lack of thyroid but was more probably due to hyper-metabolism.

In order to confirm these results a normal cow, no. 14, was fed thyroid during the fourth and fifth weeks after calving. The data presented in table 7 show no distinct rise in milk secretion. The secretion of milk fat was actually diminished in this procedure. Magee ('24) has shown that the metabolic rate per unit of weight of goats rises to a peak at the fourth week after parturition, so that the addition of thyroid at this time probably caused hyper-metabolism, which probably caused the diminution in the milk fat. It is clearly shown,

therefore, from the behavior of these two animals that thyroid feeding during rising lactation directly succeeding parturition shows little or no influence on the milk secretion. Some effects, however, may be noted on fat secretion. These effects may be related to metabolic disturbance.

TABLE 7
Cow no. 14
Daily production of milk and milk fat

DAYS	MILK SECRETION (LBS)	FAT PRODU- TION (LBS)	REMARKS
1	24.0	0.66	Calved
2	46.5	1.30	
3	47.5	1.51	
4	51.0	1.85	
5	53.5	1.87	
6	58.0	2.13	
7	59.5	2.25	
8	60.5	1.94	
9	57.5	2.07	
10	59.5	2.13	
11	60.5	2.37	
12	62.5	2.20	
13	62.5	2.26	
14	62.5	1.97	
15	59.5	1.98	
16	60.5	2.35	
17	59.5	2.00	
18	64.0	2.42	
19	59.5	2.45	
20	60.0	1.98	0.5 lb. thyroid fed daily
21	57.0	1.70	
22	61.5	2.21	
23	63.0	1.70	
24	63.0	2.15	
25	63.0	1.89	
26	63.5	1.90	
27	63.5	1.71	
28	62.0	1.61	
29	63.0	2.18	
30	64.5	2.05	
31	64.0	1.82	
32	63.0	1.93	
33	64.0	1.89	

TABLE 8

Showing the effect of the control operation on the milk secretion and milk fat production of cow no. 33

DAYS	DAILY MILK YIELD (LBS)	DAILY FAT YIELD (LBS)	REMARKS
1	27.0	1.16	Operation
2	25.0	1.11	
3	26.0	1.08	
4	26.0	1.05	
5	24.0	1.01	
6	25.0	1.00	
7	21.5	0.91	
8	18.0	0.88	
9	19.0	0.75	
10	16.0	0.62	
11	16.0	0.67	
12	13.0	0.61	
13	13.0	0.62	
14	13.5	0.56	
15	13.0	0.57	
16	12.5	0.67	
17	14.5	0.60	
18	15.0	0.60	
19	14.0	0.58	
20	13.5	0.56	
21	13.5	0.61	
22	11.0	0.54	
23	11.0	0.57	
24	11.0	0.57	
25	10.5	0.55	
26	9.5	0.44	
27	8.5	0.37	
28	9.0	0.41	
29	9.0	0.49	
30	8.5	0.33	
31	8.5	0.35	
32	9.5	0.39	
33	8.0	0.35	
34	9.0	0.37	
35	9.5	0.36	

TABLE 9

Showing the effect of the control operation on the milk secretion and milk fat production of cow no. 21

DAYS	DAILY MILK YIELD (LBS)	DAILY FAT YIELD (LBS)	REMARKS
1	19.0	0.78	
2	19.5	0.83	
3	19.0	0.81	
4	19.0	0.79	
5	19.5	0.82	
6	19.0	0.78	
7	18.5	0.74	
8	18.0	0.75	
9	18.0	0.71	
10	17.5	0.71	
11	17.5	0.69	
12	16.5	0.67	
13	16.5	0.69	
14	16.0	0.68	
15	16.0	0.64	
16	15.0	0.63	Control operation
17	13.5	0.56	
18	11.5	0.53	
19	9.0	0.51	
20	8.5	0.39	
21	8.5	0.38	
22	8.5	0.36	
23	9.0	0.35	
24	9.0	0.35	
25	10.0	0.39	
26	9.5	0.35	
27	9.5	0.37	
28	8.0	0.31	
29	7.5	0.30	Thyroidectomy
30	3.0	0.25	
31	2.0	0.10	
32	1.0	0.09	
33	1.5	0.06	
34	1.	0.10	
35	3.	0.16	
36	3.5	0.13	
37	3.5	0.14	
38	3.5	0.14	
39	3.5	0.14	
40	3.5	0.14	

SUMMARY

The removal of desiccated thyroid glands from the diet of thyroidectomized cows causes a diminution in the amount of milk and milk fat produced. Thyroidectomy itself causes a marked lowering of milk fat secretion. The diminution in milk secretion following removal of the thyroid gland cannot be readily distinguished from that accompanying control operations.

The addition of small amounts of thyroid to the diet of either thyroidectomized or normal cows, when the curve of lactation is falling, causes a rapid rise in the milk and milk fat production to a higher level after which the gradual fall, which is normal during this period in the lactation cycle, continues. During the period of rising milk secretion, which persists some 4 to 6 weeks after parturition, thyroid feeding had no apparent effect on the amount of milk secreted. Slight differences in the amount of milk fat secreted at this time may be related to the amount of thyroid fed. Feeding excessive quantities of thyroid to a thyroidectomized animal caused a diminution in milk secretion and fat secretion. From reduced milk fat percentages at this time it is apparent that the reduction in fat secretion was greater than that in milk secretion.

Lactation was not reduced to zero when thyroid was removed from the diet of the animals in the experiment. The effect of deprivation of thyroid on the total quantity of milk and milk fat produced seemed to depend on the period in the lactation cycle when the thyroid was removed.

From the results of these experiments it appears that the effect produced by the thyroid glands on milk and milk fat secretion is secondary to those factors controlling the lactation cycle. The experimental results suggest a relationship between total metabolic rate and the secretion of milk and milk fat. Fat production in these experiments appears to be more affected by the presence or absence of thyroid in the diet than is milk secretion. These rapid changes in fat secretion are thus reflected in changes in the per cent of fat in the

milk. Hence it is possible that the characteristic fluctuations in fat production, and especially fat percentage, in the records of normal cows, may be due to slight changes in the total metabolic rate of the animals.

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A COMPARATIVE STUDY OF THE SPECIFIC DYNAMIC ACTION OF THE AMINO-ACIDS ALANINE AND GLYCINE ¹

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ONE FIGURE

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The work of Lusk and his associates was preeminent in demonstrating the remarkable constancy of the specific dynamic action of certain amino-acids under carefully controlled, standard conditions. Lusk suggested that the constancy of the response entitled it to be classified with certain other physiologic constants, such as pulse rate, body temperature, blood pressure and so forth. Many careful workers in the field of nutrition agree with Lusk regarding the constancy of the response, and in view of these facts it seemed desirable to analyze the specific dynamic actions of the amino-acids, alanine and glycine, as obtained by several independent workers and to establish, if possible, a satisfactory method for expressing the specific dynamic action which would also permit comparison of experiments performed in different laboratories.

In attempting to compare the results obtained in different laboratories, several factors must be taken into consideration. Granting that the accuracy of the respective methods employed, and the care taken in training the animals are practically identical, the single most important variable factor is the

¹ An analysis of work done in the Section of Clinical Metabolism and the Division of Experimental Medicine, The Mayo Clinic, Rochester, Minnesota.

dietary regimen and the nutritional condition of the animals employed. Wilhelmj and Mann ('30) demonstrated that the specific dynamic action of the same amino-acid when administered to the same dog can be markedly altered by a period of total fasting or by total fasting followed by the administration of foods high in carbohydrate. Their experiments represent extreme conditions, but we have some evidence which appears to indicate that changes in the nutritional level at which the animals are in equilibrium can also bring about less pronounced but rather definite changes in the specific dynamic action of alanine and glycine. Because of these facts it appears necessary to limit the comparison to laboratories in which the dietary regimen and hence the nutritional conditions of the animals are approximately the same. The diet used in Rapport's laboratory and in mine was practically the same and was patterned after that described and used by Lusk. It thus appeared that the results from these three laboratories could be used in this comparative study.

The next point to be considered is the manner in which the specific dynamic actions of the same or different amino-acids can be expressed and compared. If the dogs employed are of approximately the same size and therefore have a basal heat production of approximately the same value, and if in addition, approximately the same quantity of amino-acid has been given, it is possible to compare the extra heat production during a given period after administration of the amino-acid with the basal heat production during the same period, and to express the specific dynamic action in percentage of the basal heat production. If, however, one attempts to compare the specific dynamic action in a 7-kilo dog receiving 3.7 gm. of glycine and in an 18-kilo dog receiving 9.5 gm. glycine and a 12-kilo dog receiving 30 gm. alanine and if, in addition, the duration of the respective experiments is 4 hours, 4 hours and 7 hours, the numerous variable factors become confusing and this method of comparison becomes difficult, if not impossible, to apply. The same percentage elevation may also represent a totally different actual value for the specific dynamic

action when the basal values are widely different, and since it is the actual value of the increase, in calories, that is important, this method of expression may fail to give essential and valuable information. Wilhelmj and Bollman ('28) pointed out these and other difficulties in expressing and comparing the specific dynamic actions of amino-acids, and presented evidence which appeared to indicate that the difficulties could be largely overcome by expressing the specific dynamic action as calories of 'extra' heat produced for each mol, or preferably, for each millimol, of amino-acid deaminized. Employing this method of expression I have taken a series of experiments from papers by Lusk, Rapport and Beard, Wilhelmj and Bollman, and Wilhelmj and Mann and selected experiments from the paper of Wilhelmj, Bollman and Mann, besides a series of previously unpublished experiments and have compared them on this basis.

These experiments are shown in table 1 in which fifty-one experiments are tabulated. In the seventh column the results have been expressed as calories of specific dynamic action for each millimol of alanine or glycine, deaminized, and it is apparent that the first six experiments taken from the paper of Wilhelmj and Bollman and of Rapport and Beard, although agreeing fairly well, average almost twice as high as the average values in the remaining forty-two experiments. Experiment 41 dog 3, from Lusk; experiment 11 dog 1, and experiment 3 dog 3, of previously unpublished experiments, are in poor agreement with the remaining experiments in their respective groups. These discrepancies will be referred to later; the three experiments have neither been included in the average figures for the respective groups in which they occur, nor in the total average figure.

From the results of unpublished experiments planned to investigate the higher values obtained in the experiments of Wilhelmj and Bollman and Rapport and Beard, it may be stated tentatively and with due caution that it appears that this difference is not merely chance but is of true metabolic significance, and is possibly the result of a difference in the

TABLE 1
Analysis of fifty-one experiments

INVESTIGATOR	AMINO-ACID ADMINISTERED	GRAM	NITROGEN, GM, FROM AMINO-ACID DEAMINIZED	AMINO-ACID DEAMINIZED, PER CENT	AMINO-ACID DEAMINIZED, MILLIMOL	TOTAL SPECIFIC DYNAMIC ACTION, CALORIES	SPECIFIC DYNAMIC ACTION PER MILLIMOL AMINO-ACID DEAMINIZED	LENGTH RESPIRATORY EXPERIMENT, HOURS	DOG WEIGHT, KG	BASAL HEAT PRODUCTION, CALORIES PER HOUR	REMARKS	DOG
Wilhelmj and Bollman ('28)	Glycine intravenously	5.56	0.437 ¹	42	31	15.2	0.49	4.33	10.4	21.8	Exp. 2A	
	Glycine intravenously	6.20	0.348 ¹	30	25	10.4	0.41	3.95	11.4	18.0	Exp. 5	
	Alanine intravenously	10.43	0.607 ¹	37	43	13.5	0.31	4.18	16.4	23.7	Exp. 6	
	Alanine intravenously	10.24	0.564 ¹	35	40	15.3	0.39	4.00	16.1	24.8	Exp. 1A	
Average of all = 0.40												
Average glycine = 0.45												
Average alanine = 0.35												
Rapport and Beard ('27)	Glycine orally	10.0	0.462 ²	25	33	11.2	0.34	3.00	6.5	12.4	Exp. 65	
	Glycine orally	15.0	0.652 ²	23	46	17.3	0.38	4.00	6.5	12.4	Exp. 66	
Average = 0.36												
Wilhelmj and Mann ('30)	Glycine intravenously	5.56	0.614 ¹	59	44	7.9	0.18	4.02	10.3	13.4	Exp. 2	1
	Glycine intravenously	5.56	0.478 ¹	46	34	6.3	0.19	4.12	10.4	13.6	Exp. 3	1
	Glycine intravenously	5.08	0.483 ¹	47	32	8.5	0.27	4.00	9.5	11.6	Exp. 4	1
	Glycine intravenously	5.08	0.684 ¹	72	49	10.7	0.22	4.05	9.4	12.0	Exp. 5	1
	Glycine intravenously	3.69	0.490 ¹	71	35	7.8	0.22	4.02	6.9	11.7	Exp. 1	3
	Glycine intravenously	3.69	0.547 ¹	79	38	7.4	0.19	4.02	7.0	12.1	Exp. 2	3
	Alanine intravenously	8.54	0.279 ¹	21	20	5.0	0.25	4.00	13.4	17.6	Exp. 1	2
	Alanine intravenously	8.54	0.463 ¹	35	33	5.5	0.17	4.03	13.4	16.7	Exp. 2	2
	Alanine intravenously	8.54	0.521 ¹	39	37	6.0	0.16	4.08	13.5	17.6	Exp. 3	2
	Alanine intravenously	8.54	0.623 ¹	46	44	7.1	0.16	4.05	13.6	17.8	Exp. 4	2
Wilhelmj, Bollman and Mann ('31)	Glycine intravenously	8.18	0.840 ¹	55	60	16.0	0.27	4.13	15.7	19.1		1
	Glycine orally	8.18	1.040 ¹	68	74	16.2	0.22	3.95	15.9	20.0		1
	Glycine intravenously	5.83	0.484 ¹	44	35	8.8	0.25	4.03	10.9	15.1		3
	Glycine intravenously	5.83	0.662 ¹	61	47	10.8	0.23	4.02	10.9	15.5		3
	Glycine orally	10.00	1.030 ¹	55	73	16.3	0.22	4.03	11.2	16.8		3
Average of all = 0.21												
Average glycine = 0.22												
Average alanine = 0.19												
Lusk ('12)	Glycine orally	25.0	1.98 ¹	43	144	31.3	0.22	8.00	9.6	16.2	Exp. 48	41 ⁵
Lusk ('15)	Glycine orally	25.0	2.12 ¹	45	150	28.5	0.19	8.00	9.6	16.2	Exp. 52	41 ⁵
	Glycine orally	20.0	2.38 ³	64	171	34.0	0.20	6.00	12.6	16.7	Exp. 56	3
	Glycine orally	20.0	2.53 ³	68	181	33.5	0.19	6.00	12.7	16.7	Exp. 57	3
	Glycine orally	10.0	1.29 ³	69	92	17.5	0.19	6.00	12.8	16.7	Exp. 59	3
	Glycine orally	10.0	1.25 ³	67	89	15.9	0.18	6.00	12.9	16.7	Exp. 61	3
Lusk ('12)	Alanine orally	20.0	0.801 ¹	26	58	12.6	0.22	4.00	9.7	16.2	Exp. 43	3
Lusk ('15)	Alanine orally	20.0	1.08 ³	34	76	24.0	0.32 ⁴	5.00	12.4	16.7	Exp. 41	3
	Alanine orally	20.0	1.39 ³	44	99	17.2	0.17	6.00	12.4	16.7	Exp. 43	3
	Alanine orally	30.0	2.07 ³	44	148	29.3	0.20	6.00	12.3	16.7	Exp. 44	3
Average of all = 0.20												
Average glycine = 0.20												
Average alanine = 0.20												

TABLE 1—Continued

INVESTIGATOR	AMINO-ACID ADMINISTERED	GRAM	NITROGEN, GM., FROM AMINO-ACID DEAMINIZED	AMINO-ACID DEAMINIZED, PER CENT	AMINO-ACID DEAMIN- IZED, MILLIMOL	TOTAL SPECIFIC DYNAMIC ACTION, CALORIES	SPECIFIC DYNAMIC ACTION PER MILLIMOL AMINO- ACID DEAMINIZED	LENGTH RESPIRATORY EXPERIMENT, HOURS	DOG WEIGHT, KG.	BASAL HEAT PRODU- TION, CALORIES PER HOUR	REMARKS	DOG
Unpublished	Glycine intravenously	9.47	1.060 ¹	60	76	13.8	0.18	4.05	17.7	19.6	Exp. 1	1 ⁵
	Glycine intravenously	9.47	0.939 ¹	53	67	13.4	0.20	4.05	17.7	20.1	Exp. 2	
	Glycine intravenously	9.47	0.989 ¹	56	71	15.2	0.21	4.05	17.9	20.3	Exp. 3	
	Glycine intravenously	9.47	0.953 ¹	54	68	14.2	0.21	4.07	17.7	19.8	Exp. 4	
	Glycine intravenously	9.47	1.110 ¹	63	79	13.9	0.18	4.03	17.7	20.3	Exp. 5	
	Glycine intravenously	9.47	1.120 ¹	63	80	12.9	0.16	4.05	17.8	20.7	Exp. 6	
	Glycine intravenously	9.47	0.722 ¹	41	51	11.4	0.22	4.05	18.0	20.4	Exp. 7	
	Glycine intravenously	9.47	0.774 ¹	44	55	11.2	0.20	4.05	17.9	21.0	Exp. 8	
	Glycine intravenously	9.47	0.819 ¹	46	58	9.7	0.17	4.05	18.0	20.8	Exp. 9	
	Glycine intravenously	9.47	0.872 ¹	49	62	10.0	0.16	4.03	18.2	21.6	Exp. 10	
	Glycine intravenously	9.47	0.667 ¹	38	48	14.9	0.31 ⁴	4.08	17.8	19.6	Exp. 11	
Unpublished	Glycine intravenously	5.51	0.577 ¹	56	41	8.9	0.22	4.05	10.0	14.7	Exp. 1	3 ⁵
	Glycine intravenously	5.51	0.830 ¹	81	59	13.3	0.23	4.05	10.0	14.4	Exp. 2	
	Glycine intravenously	5.51	0.837 ¹	81	60	8.2	0.14 ⁴	4.05	10.2	15.1	Exp. 3	
	Glycine intravenously	5.51	0.843 ¹	82	60	10.6	0.18	4.05	9.9	14.7	Exp. 4	
	Glycine intravenously	5.51	0.735 ¹	71	52	9.7	0.19	4.03	10.1	14.8	Exp. 5	
	Glycine intravenously	5.51	0.567 ¹	55	40	9.1	0.23	4.05	10.2	15.6	Exp. 6	
	Glycine intravenously	5.51	0.838 ¹	81	60	10.2	0.17	4.05	10.2	14.3	Exp. 7	
	Glycine intravenously	5.51	0.683 ¹	66	49	7.9	0.16	4.05	10.3	14.8	Exp. 8	
	Glycine intravenously	5.51	0.783 ¹	76	56	9.0	0.16	4.03	10.1	14.8	Exp. 9	

Average = 0.19

Average of forty-two experiments = 0.20

Average of thirty-five experiments with glycine = 0.20

Average of seven experiments with alanine = 0.19

¹ Calculated from the actual increase in urea plus ammonia nitrogen over the basal value.² Calculated from the increase in total nitrogen above the total nitrogen of the basal period. The figure thus obtained therefore includes the amount of amino-acid excreted unchanged, but since this is usually small in amount the error would not be significant.³ In comparing the increase in heat production hour by hour with the rate of deamination of the amino-acids, Lusk used percentage values obtained by Csonka based on the excretion of extra glucose in phlorhizinized dogs, and assumed that the hourly rate of deamination in these experiments would be approximately the same. However, he also gives the actual values for extra urea plus ammonia nitrogen obtained from these dogs when the same quantity of amino-acid was administered on days when the animals were not in the calorimeter. The latter, actual values, rather than assumed values have been used although there was surprisingly good agreement between the results obtained with either set of figures.⁴ Not included in averages of respective groups or in final averages.⁵ Experiments 44 and 48 as well as 41 and 52 were performed separately, but each pair can be considered as representing one experiment, since 44 and 41 cover the first 4 hours after administering 25 gm. of glycine and 48 and 52 cover the second 4-hour period, after giving the same quantity of glycine to the same dog. Each pair has been calculated as one experiment since there seems to have been in both instances a lag in elimination of extra urea during the first 4-hour period after administering glycine, and a corresponding increase during the second 4-hour period. If the experiments are calculated separately, the value of the specific dynamic action per millimol of glycine deaminized, as calculated from the elimination of extra urea, is 0.41 and 0.49 calorie for experiments 41 and 44 respectively, and 0.10 and 0.11 calorie for experiments 52 and 48, respectively.

level or plane of nutrition of the animals used. The animals used by Wilhelmj and Bollman were on a different level of nutrition from that of the remaining animals, and shortly after the level of nutrition was changed, lower figures were obtained. For the present these six experiments will be omitted from consideration in the text, and the analyses will be confined to the remaining forty-two experiments.

There was a rather remarkable constancy of the results in these forty-two experiments when expressed as calories of specific dynamic action for each millimol of amino-acid deaminized. The average value in the fifteen experiments from the papers of Wilhelmj and Mann, and Wilhelmj, Bollman and Mann is 0.21 calorie per millimol; in the nine experiments of Lusk the average is 0.20 calorie per millimol, and in the eighteen previously unpublished experiments the average was 0.19 calorie per millimol. The average value for all forty-two experiments was 0.20 calorie per millimol. The separate averages for alanine and glycine in these groups were also almost identical, and showed clearly that when expressed in this manner there was no essential difference between the specific dynamic action of these two amino-acids. A more detailed analysis of the experiments makes this constancy appear significant, in view of the following variations: 1) the weights of the dogs employed varied from 6.9 to 18.2 kilo with basal levels of heat production between 11.7 and 21.6 calories per hour; 2) the duration of the experiments varied from 3.95 hours to 8 hours, and 3) the amounts of alanine or glycine administered varied from 3.69 to 30 gm., and were given intravenously in thirty-one experiments and orally in eleven experiments. Concerning these particular factors it would be rather difficult to arrange for a much greater degree of dissimilarity.

When the relationships between the quantities of amino-acids administered, the total calories of specific dynamic action and the specific dynamic action in calories per millimol of amino-acid deaminized were analyzed, the following relations were found: 1) the maximal and minimal quantities of

amino-acid administered varied in a ratio of 1:8.1 (3.69 to 30 gm.); 2) the maximal and minimal values for the total specific dynamic actions varied in a ratio of 1:6.8 (5 to 34 calories), the low specific dynamic action of 5 calories being obtained with 8.54 gm. of alanine given intravenously and the high value of 34 calories with 20 gm. of glycine given orally, the specific dynamic actions per millimol of amino-acid deaminized in these two experiments were 0.25 and 0.20 calorie respectively (a ratio of 1:1.3), and 3) the maximal and minimal values for the specific dynamic action per millimol of amino-acid deaminized varied in a ratio of 1:1.7 (0.16 to 0.27 calorie). From this brief analysis it is obvious that widely different values for the total calories of specific dynamic action resulting from the administration of markedly different quantities of amino-acid, gave reasonably constant values when expressed as calories of specific dynamic action for each millimol of amino-acid deaminized.

The total specific dynamic action of alanine and glycine in the forty-two experiments described bears a direct linear relationship to the amount of amino-acid deaminized (fig. 1). In these forty-two experiments the quantities of amino-acid deaminized varied from 20 to 184 m.Mol. The experiments were divided into groups, each group increasing by 15 m.Mol., and the average figure in each group for the millimols of amino-acid deaminized was plotted against the corresponding average value for the total specific dynamic action. The line was constructed by multiplying the average figure for millimols of amino-acid deaminized in each group by 0.20 calorie, which is the average value of the specific dynamic action per millimol of amino-acid deaminized in the forty-two experiments. It is seen that the average values for the eight groups of experiments followed this line very closely and justified the conclusion that, within the limits of experimental error, the specific dynamic action of alanine and glycine was a linear function of the quantity of amino-acid deaminized, and that for these two amino-acids the specific dynamic action per millimol of amino-acid deaminized was approximately 0.20 calorie.

Comment

Lusk ('15) showed that the specific dynamic action of glycine is, in general, proportional to the amount administered, and further that the hours of greatest increase in heat production after giving glycine are coincident with the hours of the greatest metabolism of glycine. Chambers and Lusk

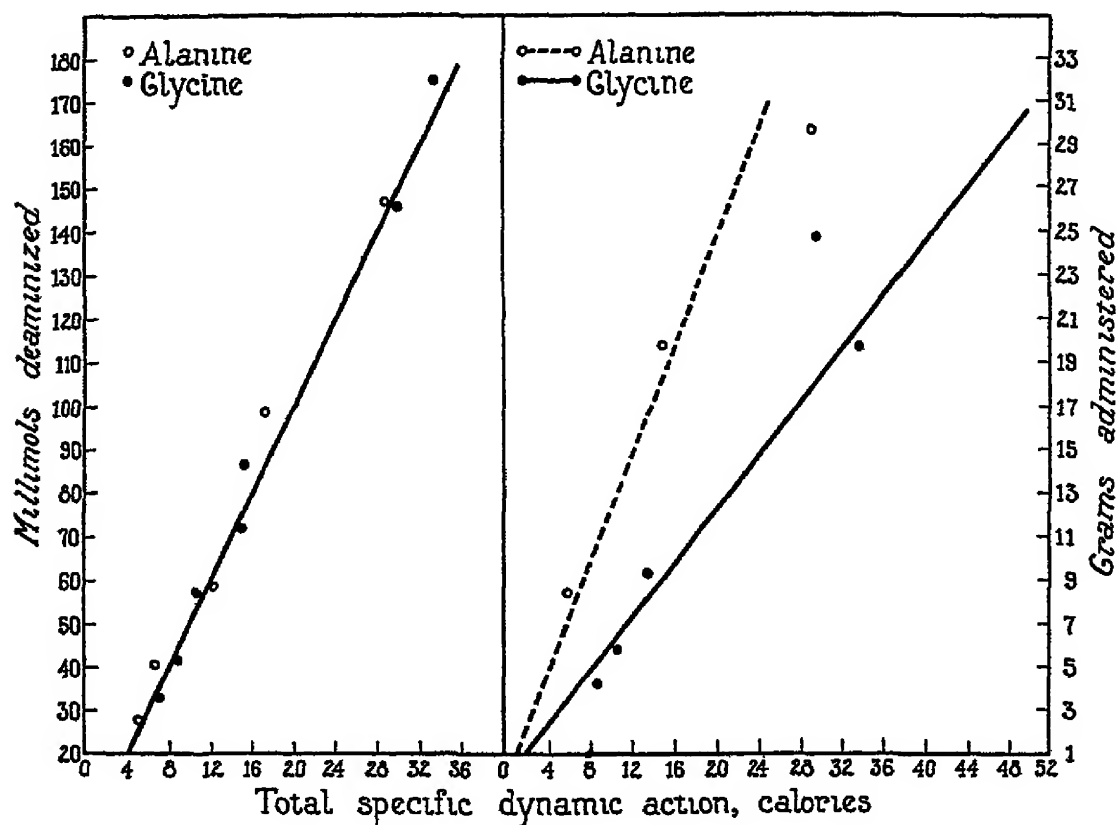


Fig.1 Chart showing the linear relationship of total specific dynamic action to the millimols of amino acid deaminized. The second figure shows the same line calculated for alanine and glycine on the basis of the number of grams administered.

('30) showed that with glycine the total specific dynamic action is independent of the size of the dog since the extra calories resulting from the ingestion of 10 gm. of glycine were the same in two dogs weighing 6.6 and 12.7 kilo. The data reported in this paper are in general a confirmation of these findings of Lusk, and they, in turn, appear to lend strength to the statement that the specific dynamic action of alinine and glycine is proportional to and can be expressed and com-

pared as extra calories of heat production per millimol deaminized. The proportionality of the total specific dynamic action to the total amount of amino-acid administered appears to depend on the quantity of the amount administered, which was deaminized; under ordinary conditions, of course, the amount deaminized increases as larger quantities are administered, but the proportionality appears to be closer when the specific dynamic action is compared with the amount deaminized rather than with the total amount given (table 1).

From the analysis recorded in this paper it appears that the specific dynamic action of alanine and glycine is practically identical when expressed as calories of extra heat produced per millimol of amino-acid deaminized. On the basis of many careful experiments Lusk, and Chambers and Lusk stated that the specific dynamic action of glycine is practically twice that of alanine, that is, for glycine approximately 100 per cent of the physiologically available calories appeared as extra heat, constituting the specific dynamic action, whereas for alanine approximately 50 per cent of the physiologically available calories appeared as extra heat of the specific dynamic action. Although these statements appear to be contradictory, a more detailed analysis shows that they are in reality the same. Lusk calculated that the physiologically available calories per gram of glycine, when combusted in the body are 2.099, and per gram of alanine 3.549 calories. Taking the molecular weight of glycine as 75 and of alanine as 89 it is found that the physiologically available calories per millimol of glycine are 0.16 and for alanine 0.32, therefore, if as Lusk found, 100 per cent of the physiologically available calories in glycine and 50 per cent in alanine appear as specific dynamic action, then when expressed as calories per millimol of amino-acid the values for alanine and glycine will be the same and will amount to 0.16 calorie. This value is very close to the average value of 0.20 calorie which was found for the forty-two experiments analyzed in table 1.

When the specific dynamic action of alanine and glycine is expressed as calories per millimol of amino-acid deaminized,

certain of the difficulties inherent in the other methods of expression appear to be eliminated and it is possible to compare the results obtained in different laboratories employing different methods, different sized dogs, and widely different quantities of amino-acids, provided certain factors, especially the nutritional conditions of the animals employed in the different laboratories, are maintained on a reasonably comparable basis. It is important to emphasize that in order to obtain such constancy of results it is essential that an equilibrium should exist between the amount of urea produced by deaminization of the amino-acid and the amount of extra urea eliminated in the urine; any factor, for instance, which produced diuresis not dependent on and proportional to that accompanying the elimination of the urea formed by deamination of the amino-acid, may by washing out urea not concerned in this equilibrium, prevent the true values from being obtained. On the other hand, any factor which reduces the ability of the kidneys to eliminate urea or increases the avidity of the tissues for holding urea, will produce errors in the opposite direction. It is possible that Lusk's experiment 41 and experiments 11 and 3 in the series of those previously unpublished, which are in rather poor agreement with the remaining experiments in their respective groups, were complicated by some such disturbance in this equilibrium. Although the values obtained in the remaining forty-two experiments are reasonably constant, it is likely that still better agreement will be obtained when other secondary factors are known and can be controlled.

There are at present no adequate explanations or hypotheses which will account for the identical value of the specific dynamic action of alanine and glycine when expressed as calories per millimol deaminized, or the constancy of the value under almost identical conditions of nutrition. At the Thirteenth International Physiological Congress, Lusk presented important data which were published by Chambers and Lusk ('30). These data were based on calculations by Adams ('26). Adams calculated that the laws of thermo-

dynamics necessitate the expenditure of certain quantities of energy in order to transform alanine and glycine into glucose and urea according to the equations of Lusk, whereas the same type of calculation when applied to glutamic acid, which was shown by Lusk ('12) and by Chambers and Lusk ('30) to exert no specific dynamic action, showed that the change to glucose and urea could be accomplished without the expenditure of energy. Adams' figures showed that to change 1 gm. of glycine to glucose and urea requires the expenditure of 0.871 calorie, whereas for alanine the corresponding value is 0.337 calorie. When these values are expressed as the energy necessary to bring about the reaction for 1 m.Mol. of amino-acid, the energy amounts to 0.067 calorie for glycine and 0.031 calorie for alanine; comparing these figures with the values for the specific dynamic actions per millimol of amino-acid deaminized in the animal body, it is seen that the actual specific dynamic action of glycine is approximately three times and of alanine approximately six times the minimal energy necessary to convert these two amino-acids to glucose and urea. Concerning these values Chambers and Lusk stated:

These are minimal and, just as external muscular work may require a three-fold energy production for its accomplishment, so this intermediary chemical work may be associated with the production of free heat. The figures given by Adams indicate a minimal energy quantum of 10 per cent of that physiologically available in 1 gm. of alanine, in order to drive it to glucose and urea, whereas the specific dynamic action of alanine is 50 per cent of the available calories in the material. In the case of glycine the minimal quantum of energy necessary to drive it to glucose and urea is 41 per cent of the available calories in the material whereas the specific dynamic action of glycine as measured by the liberation of extra heat in metabolism is 100 per cent This work of Adams explains in part, if not wholly, the specific dynamic effect of protein in the simplest way.

It is essential to emphasize that in expressing the specific dynamic action of alanine and glycine as calories per millimol of amino-acid deaminized, I do not wish to imply that the

specific dynamic action must necessarily be due to the process of deaminization per se. The studies of Lusk and his associates, which were confirmed by Aub, Everett and Fine ('27), have shown clearly that glutamic and aspartic acids may be deaminized without appreciably elevating heat production. On the other hand, the work of Wilhelmj, Bollman and Mann demonstrated that in totally dehepatized dogs, in which intravenously administered amino-acids are not deaminized, there is no specific dynamic action from alanine or glycine. These observations, in conjunction with the fact that there is a greater constancy in the specific dynamic action when it is correlated on the basis of millimols of amino-acid deaminized than on the basis of grams administered, suggests that the major part of the reactions concerned in its production occurs, under the condition of these experiments, after deaminization has taken place. In the totally fasting animal, on the other hand, there is the possibility that a certain proportion of the extra heat may arise without previous deaminization of the amino-acid, and be the result of anabolic processes by which the amino-acids are built into more complex nitrogen-containing compounds. The interesting experiments of Kiech and Luck ('31) appear to give direct evidence of such a process. Undoubtedly the intermediate steps of the metabolism of amino-acids or other substances will vary, depending on whether the animal is overnourished or undernourished, and in consequence not only is it possible for the magnitude of the specific dynamic action to vary under different nutritional conditions, but different reactions will necessarily be concerned in its production.

SUMMARY

A series of fifty-one experiments performed in three independent laboratories have been reviewed. Forty-two of these experiments appear to be comparable and have been subjected to a detailed analysis. It has been found that when the specific dynamic actions of alanine and glycine are expressed as calories per millimol of amino-acid deaminized,

the values are practically identical if animals are on approximately the same nutritional level. The average value for the specific dynamic action per millimol of amino-acid deaminized in these forty-one experiments was 0.20 calorie. The average of eight experiments in which alanine was employed gave a value of 0.19 calorie per millimol deaminized, while thirty-four experiments with glycine gave an average value of 0.20 calorie. This close agreement of the values when expressed as calories of specific dynamic action per millimol of amino-acid deaminized was obtained with dogs ranging in weight from 6.9 to 18.2 kilos and when amounts of amino-acid ranging between 3.69 gm. of glycine and 30 gm. of alanine, which gave total specific dynamic action of from 5 to 34 calories. The amino-acids were given orally in eleven experiments and intravenously in thirty-one experiments.

This analysis shows clearly that comparable results can be obtained in different laboratories employing different methods, different sized dogs and widely different quantities of amino-acids, provided that the dietary and environmental conditions of the animals are approximately the same.

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NEUROLOGICAL DISTURBANCES IN RATS REARED ON DIETS DEFICIENT IN VITAMIN A¹

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ONE PLATE (EIGHT FIGURES)

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In the course of experiments designed to study the reproductive organs in rats fed diets low in vitamin A, it was found that certain of the animals developed a marked weakness of the extremities. The weakness was manifested only in those animals which had been on the diet long enough to bring the weight increment to zero, or to develop xerophthalmia. Certain questions arose as to the relation of this condition to other symptoms of avitaminosis of A: 1) what was the incidence of the paralysis; 2) what was its temporal relation to other symptoms such as continual cornified vaginal cells, xerophthalmia and loss of weight; 3) what effect loss of weight alone has upon the nervous system; 4) what relation the age of the animal and the amount of stored vitamin have to the onset of the paralysis; 5) what neurological lesions were present; and 6) whether vitamin A was effective in curing this condition.

EXPERIMENTAL METHODS

The experimental rats were obtained from a stock in which the care and feeding had been carefully standardized. Breeding females were mated at about 100 days of age. All litters were reduced to six on the day of parturition. During preg-

¹ This investigation was partially subsidized by a grant from the National Research Council Committee for Research in Problems of Sex.

nancy and lactation the mothers of all the rats in group I and some of the rats in group III were fed 100 per cent calf-meal,² which is low in vitamin A. The other breeding females were fed table scraps, which included carrots, greens, cereals and milk, but no additional A was given.

Five groups of rats comprising forty-three animals were used. Each experimental rat was isolated in a wire-mesh cage. A wire base allowed feces to drop through. Partitions were kept between cages to prevent the spread of infection. The water bottles and tubes were washed and sterilized twice a week, and cages were sterilized weekly. Rubber gloves were worn and dipped in lysol after each animal had been handled. The animals were weighed twice a week. Fresh food was kept constantly in the cage.

It was possible to prolong the lives of rats with severe avitaminosis by keeping the room warm. The temperature was kept between 80° and 84°F. When the animals were about to die, they were sacrificed in order to preserve the tissues while fresh and each was autopsied. The gross pathological lesions were recorded, and the tissues prepared for microscopic study. The brachial plexuses, sciatic and vagus nerves were dissected out and fixed in Müller's fluid and in 10 per cent formalin. The spinal cords and brain were fixed in 95 per cent alcohol. Dr. Harry M. Zimmerman ('33) studied these materials; the result of his observations which have been published need be only briefly summarized here. His diagnosis is based on sections stained with the original Nissl method, Spielmeyer's stain, Scharlach R and osmic acid.

² Calf-meal is a commercial mixture containing approximately the following ingredients in 100 parts; oil meal 15, malted barley 10, whole wheat 22, oat flour 15, dried skim milk 15, yellow corn meal 20, ground limestone 1, steamed bonemeal 1, salt 1.

Diet. The composition of the vitamin A deficient diet was:

<i>Ingredient</i>	<i>Per cent</i>
Casein ³	15-18
Cornstarch	56-63
Hydrogenated vegetable oil ⁴	18-22
Osborne-Mendel salt mixture IV	4

Because rats ate more when the food was varied the proportions of the diet were changed weekly or biweekly. This was done in the following manner; casein, cornstarch and hydrogenated vegetable oil were changed from 15, 63, 18 per cent to 18, 56, 22 per cent, respectively.

Adequate amounts of vitamins B, D and E were supplied by yeast,⁵ irradiation,⁶ and hydrogenated vegetable oil. Vitamin C has not been found necessary in the normal metabolism of the rat (Parsons, '20). The tests of the yeast showed that $\frac{1}{10}$ gm. daily was sufficient to produce a gain in weight in rats deprived of vitamin B. The same batch of yeast was used throughout the experiment. Four to eight-tenths of a gram was fed daily to these experimental animals in separate containers. In addition, tiki-tiki⁷ and yeast concentrate were fed to some of the rats. A biological test of ergosterol showed that 0.0009 mg. fed over a period of 10 days gives a 2-plus line test. One-thousandth to two-thousandth gram irradiated ergosterol was given daily, if no irradiated yeast was included. With the irradiated yeast it was sometimes omitted. To irradiate the yeast it was spread in thin layers, exposed to the rays of a mercury-vapor lamp at a distance of 15 inches for 15 minutes, then mixed, re-spread, and irradiated for another 15 minutes (Hess, '27).

Evans and Burr ('27) were able to obtain successful reproduction in rats on an E-free diet, by giving 22 per cent of hydrogenated vegetable oil⁸ in the diet or by feeding 500 mg. of the same oil during the gestation period.

³ Eimer and Amend technical casein.

⁴ Crisco.

⁵ M. H. Givens, of the Northwestern Yeast Company, supplied tested yeast.

⁶ Irradiated ergosterol was supplied by the Fleischmann laboratories.

⁷ The author is indebted to Dr. George R. Cowgill for supplying tested tiki-tiki and yeast concentrate.

⁸ Crisco.

Frequent observations were made in order to record the first appearance of vitamin A deficiency. Vaginal lavage was taken on each rat four to six times a week. The appearance of continued cornified cells in the vagina was found to be the first indication of vitamin A deficiency (Aberle, '33). The rats were watched with similar frequency for the appearance of xerophthalmia or loss of weight. The diagnosis of xerophthalmia was based upon the appearance of red granules in or about the eyes. The failure to gain weight was taken when the weight increment was zero. Incontinence of urine was recorded when it was first noted that the hair about the urinary papilla of the rat was wet and stained. After this, a constant dribbling of urine and a characteristic odor were present.

RESULTS

Group I. Rats on diets deficient in vitamin A, from mothers on diet of 100 per cent calf-meal during pregnancy and lactation. The average age at which they were weaned, and the time of the various manifestations of A deficiency are shown in table 1. The first appearance of the paralysis in this group occurred at the average age of 61.1 days. The symptoms of the paralysis were arbitrarily divided into four stages, in order to facilitate record taking. Each phase was defined as having one outstanding characteristic, although there was variation in the duration and severity of the symptoms.

Stage I showed best when the animal was suspended by the nape of the neck (fig. 2). There was a hyper-extension of the hind legs and a marked curvature of the spine. In the normal rat (fig. 1), the legs are usually flexed when they are removed from the floor. If flexion does not occur normally, it can be induced by stimulation to the sole of the foot. In the rats in stage I slight stimulation such as scraping the sole of the foot with the blunt part of forceps was not sufficient to cause the legs to be flexed. The rat shown in figure 3 had stages I and II of the paralysis. During stage I the legs of the experimental rats spread farther apart in walking than those of the normal animal and there was an awkwardness in the movements.

TABLE 1

Age and weight¹ of rats at the appearance of various symptoms of avitaminosis of A

	WEANED AND DIET STARTED	CONTINUAL CORNIFIED SMEAR	XEROPH- THALMIA	WEIGHT- GAIN STOPPED	INCOON- TINENCE	PARALYSIS STAGE ²				O L O.	DIED	KILLED	PARALYSIS DIS- APPEARED
						I	II	III	IV				
Group I. Rats on vitamin A deficient diet from mothers on diet of 100 per cent calf meal during pregnancy and lactation													
Number of rats	12	12	10	10	8	9	5	8	4		6	6	
Mean age (days)	22.0	46.5	58.6	64.1	68.1	61.1	62.6	66.8	81.8		78.3	74.7	
S.D.M.	0.3	2.0	0.8	3.0	3.5	1.8	2.3	4.4	2.1		4.5		
Mean weight (grams)	28.3	75.3	88.4	93.4	86.3	87.7	83.6	84.6	89.8		85.7	86.0	
S.D.M.	1.2	4.6	5.5	4.6	4.8	4.5	5.5	4.6	5.4		5.8		
Group II. Rats on vitamin A deficient diet from mothers on table scrap diet													
Number of rats	15	10	9	7		6	6				3	12	
Mean age (days)	21.6	62.7	105.1	111.0		112.7	121.5				75.3	84.9	
S.D.M.	0.2	2.2	3.5	3.5		2.0	2.5				65.6	108.1	
Mean weight (grams)	35.4	124.5	147.0	156.4		159.7	153.3						
S D M.	1.8	8.4	9.9	10.5		11.2	13.1						
Group III. Rats allowed to develop neural symptoms and then given cod liver oil													
Number of rats	6	6	5	5	2	1	5	2	2	6	1	5	3
Mean age (days)	21.8	55.3	63.0	62.8	58.5	56.0	68.4	67.5	71.0	76.8	90.0	97.0	96.7
S.D.M.	0.3	3.3	1.9	2.3	77.5	60.0	2.1	81.0	79.0	1.7	60.0	149.2	160.0
Mean weight (grams)	31.5	91.2	95.8	100.4			94.4			88.7			
S.D M.	1.3	9.3	11.2	10.5			9.0			7.5			

¹ Weight on nearest day, ± 1 to 4 days.

² Stage I. Failure to flex hind legs upon suspension Stage II. Legs slip in walking Stage III. Sprawling walk. Stage IV. Dragging hind legs.

The next symptoms, characterizing stage II, were noticed when the animal walked. These symptoms appeared at an average age of 62.6 days in this group. There was incoordination of movements. The hind feet, instead of being placed in one position which was maintained until the foot was lifted to take the next step, slipped at each step (figs. 3, 4). When placed in a position to take a step, however, the foot was approximately parallel to the body.

The outstanding symptom for stage III appeared at an average age of 66.8 days. The feet, instead of being held parallel to the body, were extended at an angle of about 45° in standing and in walking (fig. 5). The helplessness of the hind legs can best be seen in the photograph showing a rat with stage II paralysis trying to climb a small wire fence (fig. 8). The hind legs are so weak that they can scarcely be moved. Some of the animals showed a marked degree of spasticity. The control climbed over the fence easily (fig. 7).

Four animals lived to show stage IV of the paralysis. At this time the legs were dragged along helplessly when the animal moved forward (fig. 6). The fore legs showed involvement in only one animal. In general, clumsiness and incoordination appeared first; then, if the condition progressed further, spasticity appeared. Whether or not the sensory loss occurred in the order of muscle sense, touch and pain, cannot be determined from the observations made.

Of the twelve rats from mothers on a calf-meal diet, two died before and one after xerophthalmia appeared. The remaining nine all developed the paralysis. This remained the same or became progressively worse on the A deficient diet. Paralysis occurred at about the same time as xerophthalmia or loss of weight. The clinical symptoms of the paralysis were confirmed by microscopic sections of the cord and peripheral nerves. The traces of vitamin A present in technical casein probably were responsible for their survival after symptoms of avitaminosis had become marked. Five of these animals were given additional tiki-tiki and yeast concentrate, but the extra vitamin B had no effect on their symptoms.

In each of the nine rats lesions were found in the spinal cord consisting of degeneration of the medullary sheaths of the sensory tracts at the periphery. In four of the animals the posterior columns showed degeneration; and in two, the entering posterior nerve roots showed it. These changes were demonstrated by the Spielmeyer myelin-sheath stain and by the Marchi method. In rat 72, paralyzed for the longest period, fatty change was demonstrated in the white matter of the cord by the Scharlach R stain. In rat 65 there was degeneration of the anterior roots in addition to the posterior. The vagi were degenerated in only four of the animals examined; in one they were not examined. Every one of the animals showed extensive degeneration⁹ of the medullary sheaths of the brachial plexuses and sciatic nerves.

Group II. Rats on diet deficient in vitamin A from mothers fed a table scrap diet. Although the breeding females were not given additional vitamin A in the form of cod liver oil, they probably had enough to endow the offspring with a considerable store of the vitamin.

Of the nine rats who lived to develop xerophthalmia, six showed stage I or II, or both, of the paralysis. Although at that time their average age and weight were approximately twice that of those in group I, no further symptoms developed. The paralysis did not appear as early, or with the same intensity as group I, which started without as much stored vitamin A in the tissues. The lesions of the peripheral nerves of two animals which were examined, were similar to those in group I. The cord of one rat was studied and found to show degeneration of the posterior columns and nerve roots, the motor tracts and to a less degree the anterior roots.

Group III. Six rats allowed to develop neural symptoms and then given cod liver oil. Two rats came from mothers which had been on 100 per cent calf-meal during pregnancy and lactation. These rats developed all four stages of the paralysis and incontinence of urine. Tiki-tiki or concentrated yeast had no effect on the symptoms. After the symptoms

⁹ These lesions are often designated by the term polyneuritis in the literature.

were well established, cod liver oil was administered daily. In one rat, to which cod liver oil was given on the sixty-fifth day of life, xerophthalmia completely disappeared within 12 days, incontinence of urine in 5 days, and the neural symptoms by 90 days of age, or 25 days after cod liver oil therapy. At this time the rat's weight was almost double that at the time therapy was initiated.

In the second rat after xerophthalmia was marked and all the stages of the paralysis were well developed, cod liver oil was given. Eight days after daily feeding of cod liver oil the eyes had cleared up. Thirteen days later, when the animal was killed, the paralysis had improved from stage IV to a II condition.

Three rats (from mothers on a table scrap diet until the thirteenth day of lactation, then placed on 100 per cent calf-meal) developed only stage II of the paralysis. After they had showed symptoms for 4, 5 and 10 days, they were given cod liver oil. Clinical symptoms of the paralysis disappeared after 20 and 22 days in two animals, and after 14 days in one rat which died. One rat from a mother on a table scrap diet did not develop any symptoms of the paralysis.

In the four animals where clinical recovery occurred, and in which the spinal cords and peripheral nerves were examined microscopically, it was found that they contained extensive lesions, essentially the same as those found in group I. The clinical improvement, therefore, must be due entirely to repair in the tissues not demonstrated by the technique used. This clinical improvement with neural lesions shows the possibility of having no demonstrable clinical symptoms in the presence of extensive degeneration within the central nervous system.

Group IV. Rats kept on a table scrap diet, then given a limited amount of food. Four rats were kept on a table scrap diet until 50 days of age. They were then given 1 gm. daily of the purified food and vitamins as given to the rats on the A deficient diet, and in addition $\frac{1}{2}$ cc. of cod liver oil. Their loss of weight on this restricted diet paralleled that of the rats on the A deficient diet. They died from 10 to 17 days after

being put on the experimental diet, mean weight 64.5 gm. They showed no symptoms of paralysis. They were weak and exceedingly irritable and had rough coats, but the feet were held in the usual position, both when the rats were extended by the nape of the neck and when they walked. In two animals the nervous tissue was examined. No degeneration was found in the cords. All the peripheral nerves were free of degeneration except for one small zone in the nerve of one animal where, by the use of Marchi preparations, a few black granules were found. In group I the average life of two of the three rats, which showed clinical or microscopic evidence of the paralysis, was 13.5 days after they commenced losing weight. This length of life shows that the loss of weight alone did not account for the paralysis.

Group V. Controls. The control group contained a total of six animals, five from mothers on table scrap diets and one from a mother on 100 per cent calf-meal during pregnancy and lactation. Four were given the purified diet with cod liver oil, and two were kept on table scraps. In none of these animals were signs of the paralysis observed clinically. In one animal from a mother on 100 per cent calf-meal during pregnancy and lactation, and in two rats given the table scrap diet, a complete investigation of the nervous system, central and peripheral, by the histological methods used in this study, failed to reveal any degeneration. The fat content of the purified diet had apparently no deleterious effect on the nervous system.

It is evident that both the purified diet when supplemented with cod liver oil and the table scrap diet when given over a long period of time were capable of maintaining the rats in a satisfactory state of nutrition, without clinical or microscopic lesions of the nervous system.

PATHOLOGY

The gross lesions found at autopsy in the various groups were as follows: of the twelve rats in group I, six showed foci of infection in the kidneys; seven showed bladders filled with

pus; two showed lung infections; six showed infections of the glands at the base of the tongue. In group II the infection was less. Of ten animals examined there were found only one kidney lesion, two lung lesions and five infections of the tongue. Of six animals examined from group III, only one kidney and one tongue lesion were found. In groups IV and V no foci of infection were found. No salivary gland lesions were found on gross examination in any of the rats. Many of the tissues were checked by histological examination. No lesions were found on section of nine intestines, four stomachs, three livers, all taken from the most depleted animals in groups I and II. Infection was found in the tracheal glands of eight of the experimental animals examined. Necrosis was found in the vagina of three rats out of a total of fifteen examined histologically. The bone marrow from seven of the most depleted rats was not histologically different from that of the control.

DISCUSSION

In the group of rats dealt with in this experiment certain neurological symptoms with lesions of the nervous system developed from a lack of adequate vitamin A. These appeared only in those animals suffering from a long continued deficiency. In the acute cases the animals died before the paralysis was manifested. In the group where twelve animals started with little or no stored vitamin (group I), nine of the animals which lived beyond the time when severe avitaminosis was present exhibited clinical symptoms of the paralysis. All these rats showed evidence of neurological lesions.

The fifteen rats which started the experiment with some storage of vitamin A (group II) were heavier at weaning and throughout the experiment. Ten lived to show continual cornified smears, eight exhibited stage I or II, or both of the paralysis, and none had further symptoms of neural lesions. The first symptom of the paralysis appeared about 7 weeks later than the paralysis in the group which started the experiment without a storage of vitamin A. In the rats which

started the experiment without a storage of the vitamin the paralysis affected more animals and appeared more quickly, the symptoms were more pronounced, less time elapsed between symptoms, and death occurred sooner than in the rats which started the experiment with a storage of the vitamin. The symptoms of paralysis always occurred after the appearance of continual cornified vaginal cells and at about the same time or somewhat later than the xerophthalmia or loss of weight. The incidence, the severity, and the progression of the neural symptoms were shown to depend directly upon the amount of vitamin A available, either stored or ingested (Aberle, '31).

The paralysis did not appear in animals suffering severe inanition with plenty of vitamins (group IV), even though their weight approximated that of rats with severe avitaminosis. An examination of their nervous tissue confirmed the clinical diagnosis. At death the weight of the animals with a restricted diet averaged only 2 gm. less than that of the rats with stage II neural symptoms.

Anaemia was probably not a contributory factor. Turner ('30) has shown that rats on diets deficient in vitamin A do not show a difference in the non-protein nitrogen, creatinine, urea nitrogen, chloride, sugar, or red blood corpuscles when compared to the controls. In the bone marrow of rats examined microscopically, no difference could be ascertained between the experimental and control animals.

Although the symptoms resemble those described by Cowgill ('21) for dogs suffering from lack of vitamin B complex, yeast had no effect upon preventing, alleviating, or curing the disorder. All the rats received large daily feedings of yeast. In the seven animals where additional B was given in the form of concentrated yeast or tiki-tiki, no change either clinically or microscopically was noted.

Control rats on the purified diet with cod liver oil never developed neural disturbances clinically or histologically. Vitamin C was absent in both experimental and control groups, which would eliminate its importance as a factor re-

sponsible for the paralysis. The control rats were given the same proportions of the purified diet as were the experimental rats. Thus, the fat content of the diet was of no significance as far as the neural manifestations were concerned (Burr and Burr, '29). Four rats were cured clinically by administering cod liver oil. Their nervous tissue still showed lesions. When recovery takes place the tissue resembles tissue from the more acute pathological stages, but the physiology is different. In the opossum, Weed and Langworthy ('25) and Langworthy ('28) found that it was possible to stimulate the motor cortex and elicit movement in the fore legs 41 days before the cortico-spinal tract myelinated. Myelinization is not the exact criterion it has been supposed to be. It is not impossible that the axis cylinder may function before the myelin is redeposited. It might be that the axis cylinders in many cases were interrupted at a certain stage of avitaminosis and that later after resuming vitamin A feeding, the axis cylinder was again continuous.

Lesions of the central nervous system due to avitaminosis of A are suggested by investigations dealing with dietary deficiencies which were reported some 19 years ago. Hart and McCollum ('14) reported the possibility of a 'toxic substance' in diets containing over 90 per cent of wheat. The ill effects of the diet were manifested in a loss of weight, rough coat, emaciation, lack of coordination of the muscles, and partial blindness. The conditions were found to be improved by the addition of butter fat.

Later, Hart, Miller and McCollum ('16) fed swine a diet low in vitamin A. After 9 months, growth ceased, followed by loss of weight, difficulty in locomotion, rough coat, labored breathing and muscular twitching. In some animals extreme stiffness and dragging of the rear quarters was evident. The investigators came to the conclusion that "malnutrition, histologically characterized by nerve degeneration, may result from the absence of certain factors in the diet as in the case of beri-beri. A similar condition may likewise arise from the presence of toxic materials in apparently normal food products"

Mellanby ('26, '30, '31) reported experiments in which puppies fed a diet deficient in fat soluble vitamins developed symptoms associated with nerve lesions. The most outstanding symptoms were severe incoordination, spasticity and weakness. The spinal cord of the animals, stained by Marchi's method, showed a scattered degeneration of the nerve fibers. Mellanby ascribes the condition to the 'toxic' effect of a large amount of cereal, other than yellow corn, in the diet.

On the other hand, Steenbock, Nelson and Hart ('21, '22) describe a peculiar gait in dogs on a diet deficient in vitamin A. No description is given of the lesions. Hughes et al. ('28, '29) reported nerve lesions in pigs, chickens and cows, due to lack of vitamin A. The symptoms were characterized by impaired vision, incoordination and spasms. They also describe the arching of the pig's back while standing or walking. This condition was noted in most of the rats and described under stage I of the paralysis. Histological examination of the nerves of the pigs in advanced stages of avitaminosis showed degeneration of the nerve bundles in portions of the spinal cord, and in the optic, sciatic and femoral nerves. No 'toxicity' is postulated for these experimental lesions. Suzman, Muller and Ungley ('32) attempted without success to produce lesions in the spinal cords of adult dogs by feeding a diet abundant in cereals but lacking in vitamin A. Their failure to obtain symptoms of avitaminosis was probably due to the age of the animals and the amount of stored vitamin with which they started the experiment. The lesions described by all of these investigators are somewhat similar and both clinically and histologically resemble those found in the rats here reported, clumsiness, incoordination, weakness and later on spasticity being the outstanding symptoms. In this experiment no cereal was fed. Before 'toxicity' of certain foods could be postulated, it would be necessary to feed animals on a diet adequate in vitamin A.

CONCLUSIONS

A disabling paralysis has appeared in rats suffering from chronic deficiency of vitamin A. The paralysis was characterized clinically by clumsiness, incoordination and finally a spasticity, with a complete lack of control of the affected limbs. Microscopical examination showed the paralysis to be associated with degeneration of parts of the nervous system.

The paralysis did not appear in control animals kept under identical experimental conditions, and fed the same purified foods, except for the inclusion of cod liver oil. It did not appear in rats on a table scrap diet, or in rats with severe inanition but provided with plenty of vitamins.

The paralysis was closely associated with other symptoms of avitaminosis. Neurological symptoms were always preceded by the continuous appearance of cornified vaginal cells, and were frequently preceded by xerophthalmia and loss of weight.

The incidence of paralysis among rats which at the beginning of the experiment were without stored vitamin A was greater, they exhibited the paralysis sooner, they had more pronounced symptoms, and they showed less time between symptoms and died sooner than rats which had come from parents whose vitamin A storage had been ample.

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PLATE 1

EXPLANATION OF FIGURES

1 Control rat, showing characteristic flexion of the hind legs when held by the loose skin at the nape of the neck.

2 Rat showing stage I of the paralysis; a hyperextension of the hind legs and marked curvature of the spine when held by the loose skin at the nape of the neck. Nineteen days following the first symptom of vitamin A deficiency and 58 days on purified diet.

3 Rat showing stage II of the paralysis. The left foot slipped when walking. Nineteen days after first symptom of vitamin A deficiency and 58 days on purified diet.

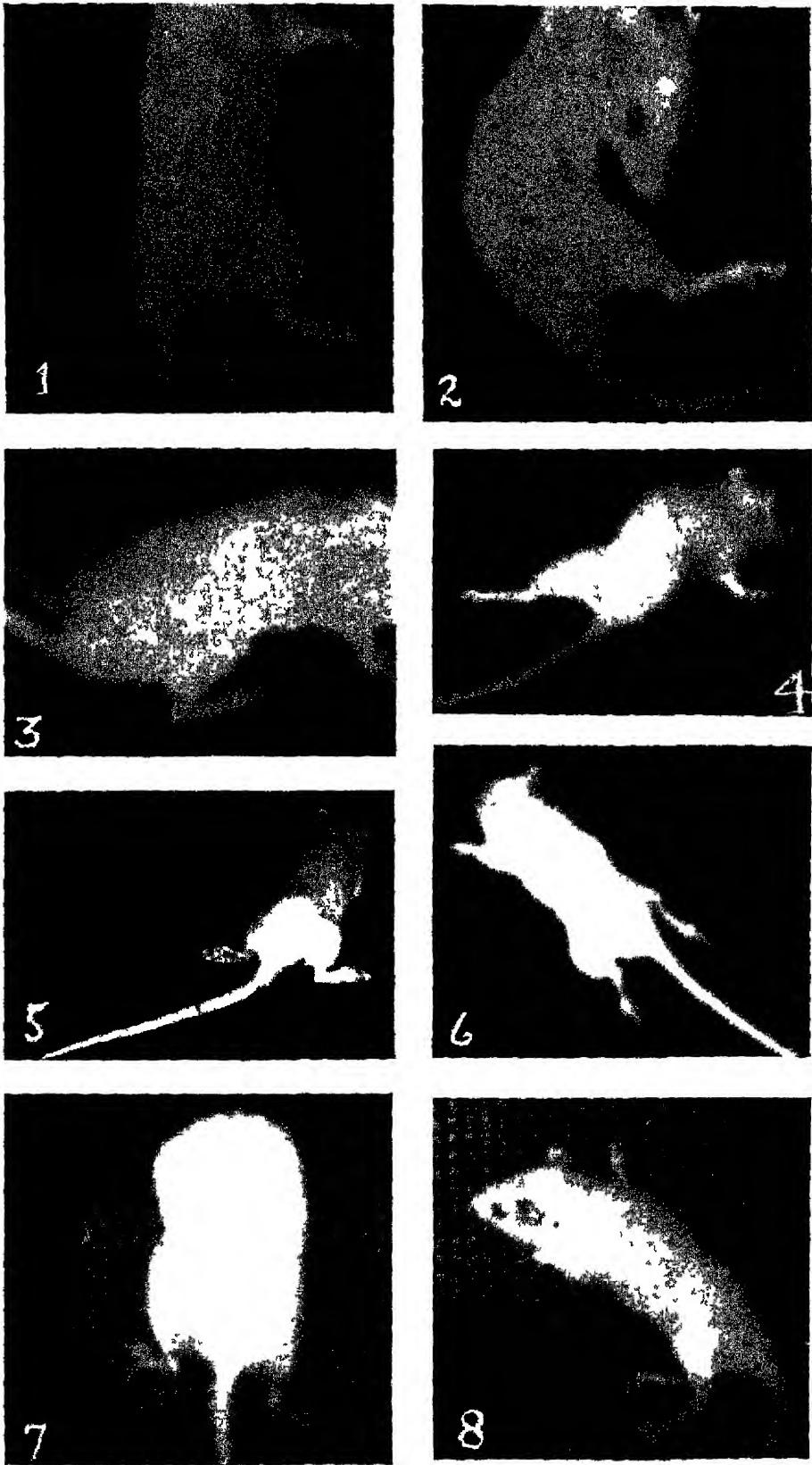
4 Rat showing stage II of the paralysis. The left leg has slipped away from the body, the right leg is beneath the body of the rat. Ten days after the first symptom of vitamin A deficiency and 45 days on purified diet.

5 Rat showing stage III of the paralysis. The hind legs are held out from the body and the feet are at about a 45° angle from the long axis of the rat. Forty-three days on purified diet and 18 days after first symptoms of vitamin A deficiency.

6 Rat showing stage IV of the paralysis. The front legs are used for locomotion while the hind legs are drawn helplessly along. Forty-three days on purified diet and 13 days after first symptom.

7 Control rat climbing over a wire fence. The rat had been 95 days on purified diet with cod liver oil.

8 Rat attempting to climb wire fence. Animal has been on the purified diets for 53 days, 18 days after the first symptom of vitamin A deficiency.



RELATION BETWEEN THE PHYSICAL CHARACTER OF FOOD AND DENTAL CARIES IN ALBINO RATS

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TWO FIGURES

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There is no agreement as to the etiology of dental caries. Excess of carbohydrate, *Bacillus acidophilus*, deficiencies of vitamins A, B, C, and D, as well as minerals have all been suggested as causative factors. More recently the physical character of the dietary constituents has been accused.

We have reported that different diets containing 73 per cent of starch, 66 per cent of glucose, 66 per cent of lactose and 66 per cent of maltose failed to produce dental caries in 6½ months (Lilly and Grace, '32) and that triweekly inoculations with the *Bacillus acidophilus* of the mouths of rats for a year was not provocative of dental caries even in animals subsisting on high carbohydrate diets for a year (Lilly, '32). We also attempted to produce dental caries by placing rats on diets deficient in vitamin D. These animals were kept in a dark room on Steenbock's Rachitogenic Diet no. 2965 and distilled water, and received no added vitamin D. Some of these animals lived 6 and 7 months and two survived 11 and 12 months respectively, yet no caries developed in any of these animals.

In addition, avitaminosis and slow starvation produced no caries in rats under our observations, since Mendel's normal rat diet made up with deficiencies of either vitamins A, B or

D (rats synthesize vitamin C) produced no caries up to the death of those animals. Furthermore, other diets so low in protein and minerals that only one-half the size and weight and one-fourth the reproduction of normal rats were attained, produced no dental caries in three successive generations.

In several hundred autopsies on rats fed either on table scraps or Mendel's normal rat ration, in this laboratory, not a single instance of dental caries has occurred.

These observations made it evident that there was nothing inherent in the routine management of our animals that produced dental caries.

The experiment about to be reported deals with the idea suggested by Hoppert, Webber and Canniff ('31, '32) who report the occurrence of dental caries in relation to 'particle size' and 'retention particles' of cornmeal-fed albino rats.

Klein and McCollum ('31), in a comment, attributed the caries reported by Hoppert, Webber and Canniff to a phosphorus deficiency of the caries producing diet.

The basic diet used in our experiment was as follows:

	<i>Per cent</i>
Yellow cornmeal,	60
Whole dry milk ¹	30
Flaxseed meal	6
Alfalfa meal	3
NaCl	1
	<hr/>
	100

¹ Klm

This is a stock ration used by many investigators for normal rat colonies.

To test the efficiency of this ration on our strain of rats, we fed a colony of our animals this diet through four generations and found it maintained an average of size, weight, and reproduction comparable to that produced by Mendel's normal rat diet.

This diet having proved adequate according to present standards was then varied as follows; however, one variable only was permitted in each test.

Series I. The basic diet was made up by using yellow cornmeal from a nearby grocery store. This was a fine meal, as commercial meal is screened before being put on the market.

Thirty-five rats, 26 to 31 days old, from our own colony were fed this diet for 200 days. The animals were then chloroformed, the heads cleaned, and the teeth examined under magnification but no caries was present.

Series II. Yellow corn was then coarsely ground in a cornmeal mill, and the whole product collected. This product was then sifted in a 60-mesh standard sieve; about one-third of the meal passed through the sieve, while about two-thirds did not. Thus a fine and a coarse meal was obtained.²

Group 'A.' The basic diet was then prepared by using the meal that did not pass the sieve.

Twenty-four rats, 27 to 30 days old, from our own rat colony were fed this diet for 100 days. The animals were then chloroformed, the heads cleaned, and the teeth examined under magnification for caries when fifteen of the twenty-four rats or 62 per cent were found to have from one to three large carious lesions in the molar teeth.

Group 'B.' The basic diet was prepared by using the meal that passed the sieve.

Twenty-four rats, 27 to 30 days old, from our own colony were fed this diet for 100 days. The animals were then chloroformed, the heads cleaned and the teeth examined under magnification for caries but no caries was found.

Group 'C.' The basic diet of this group was identical to the diet fed group A, except that the meal was ground through a 'hammer mill' before being mixed with the other ingredients of the diet. The hammer mill³ reduced the meal only sufficiently for two-thirds of it to pass the 60-mesh sieve.

Twenty-four rats, 25 to 30 days old, from our own colony were fed this diet for 100 days, when the animals were chloro-

² The sifting is a tedious process, a stiff brush being needed to express the fine portion through the sieve.

³ We are indebted to the Chelsea Flour Milling Company for the use of the hammer mill.

formed, the heads cleaned, and the teeth examined under magnification for caries. Five of the twenty-four rats (or 20.8 per cent) had one or more caries.

Group 'D.' The basic diet of this group was identical to that fed group 'A' except that $\frac{3}{10}$ per cent viosterol (250 D) by weight was incorporated into the diet.

Fifteen rats, 25 to 30 days old, from our colony were fed this diet for 100 days, then chloroformed, the heads cleaned, and the teeth examined under magnification for caries. Eight of these fifteen rats (or 53 per cent) had one or more dental caries.

Series III. A new lot of yellow corn was coarsely ground and the whole product collected and sifted in a 60-mesh sieve and a fine and a coarse meal obtained exactly as in series II.

Group 'A.' The basic diet was prepared by using the meal that did not pass the 60-mesh sieve.

Sixteen rats, 25 to 30 days old, from our own colony were fed this diet for 125 days (25 days longer than in series II). The animals were then chloroformed, the heads cleaned and the teeth examined under magnification for dental caries. All of the animals (100 per cent) had one to three large carious molar teeth.

Group 'B.' The basic diet was prepared by using the meal that passed the 60-mesh sieve.

Ten rats, 25 to 30 days old, from our own colony were fed this diet for 125 days. The animals then were chloroformed and examined for dental caries as previously described but no caries was present.

Series IV. A new lot of yellow corn was coarsely ground, the whole product collected, sifted in a 60-mesh standard sieve, and a fine and a coarse meal obtained exactly as in series II and III.

Group 'A.' The basic diet was prepared by using the meal that did not pass the 60-mesh sieve.

Nine rats,⁴ 25 to 30 days, old, from our own colony were fed this diet for 100 days. The animals were then chloro-

⁴ This group was fed as a 'control' for this lot of corn, and found productive of caries

formed and examined as previously described for dental caries.⁵

Four of the nine rats (or 44 per cent) had one or more carious molar teeth.

Group 'B.' The diet for this group was identical to the diet fed group 'A' except that the meal was cooked for 2½ hours in a double boiler under distilled water before it was mixed with the other ingredients of the diet.

Sixteen rats, 25 to 30 days old, from our own rat colony were then fed this diet for 100 days, then chloroformed and examined as previously described for dental caries but no caries was present.

Group 'C' The diet for this group was identical to the diet of group 'A,' except that the meal was ground through a Schultz-O'Neil mill⁶ before it was mixed with the other ingredients of the diet.

The Schultz-O'Neil mill ground the meal fine enough to pass a 60-mesh sieve. However, a very small portion of a chaffy material did not pass the sieve; this was reduced by mortar and pestle until it did, then put back into the diet.

Sixteen rats, 25 to 30 days old, from our own colony were fed this diet 100 days, then chloroformed and examined for dental caries as previously described but no caries was present.

Calcium and phosphorus metabolism

Calcium and phosphorus balances were obtained for 5-day periods during the preceding experiments for the finely ground corn and the coarse meal, both cooked and uncooked. In each experiment the rats were placed together in a large cage and the food carefully weighed. At the end of the experiment the cage was carefully cleaned and washed, all of the excreta and washings being placed in a large round-bottomed flask. Any food which had been dropped in the cage

⁵ Ten animals were started on this test, one died at the end of the first month of the test

⁶ We are indebted to Frederick Stearns & Co., of Detroit, Michigan, for the use of their Schultz-O'Neil mill

was included with the material in the flask. The contents of the flask were evaporated to dryness in an oven, and digested for several hours with concentrated hydrochloric acid under a reflux condenser. The material was then centrifuged and washed three times with water, and the liquid obtained by centrifuging and the washings was placed in a 2-liter volumetric flask. The precipitate was returned to the digestion



Fig. 1 Microphotograph of a typical dental caries in a lower molar of an albino rat fed on the coarse cornmeal diet for 100 days

Fig 2 Enlarged photograph of lower jaw of rat fed the coarse cornmeal diet for 100 days, showing typical dental caries.

flask and the process repeated until the precipitate in the centrifuge tubes gave no test for calcium or phosphorus. The combined liquors and washings were made up to volume in the 2-liter flask.

Aliquot parts of this solution and the diets were analyzed for calcium by the method of Tisdall and Kramer ('21) and for total phosphorus by the method of Fiske and Subbarow ('25).

The results of these experiments are shown in table 1. Since the food which was lost in the cage was included with

TABLE 1
Calcium and phosphorus balances

DIET	NUMBER OF RATS	CALCIUM			PHOSPHORUS		
		In	Out	Balance	In	Out	Balance
		<i>mg.</i>	<i>mg</i>	<i>mg./rat/day</i>	<i>mg.</i>	<i>mg</i>	<i>mg./rat/day</i>
Coarse	7	1285	899	+11.3	1750	1250	+14.3
Coarse	3	655	576	+ 5.3	883	714	+11.3
Coarse cooked	7	1260	1220	+ 1.1	1670	1620	+ 1.4
Fine	6	1089	939	+ 5.0	1245	1110	+ 4.5
Fine	1	245	232	+ 2.6	268	217	+10.2

the excreta in the determination of calcium and phosphorus, this factor does not influence the estimation of the balance for these two elements. The balances were calculated per rat per day. Two experiments on the coarse uncooked diet gave calcium retentions of 11.3 and 5.3 mg. and phosphorus retentions of 14.3 and 11.3 mg. respectively. One experiment on the cooked coarse diet indicated that the rats were practically in calcium balance. Two experiments on the fine diet gave calcium retentions of 5.0 and 2.6 mg. and phosphorus retentions of 4.5 and 10.2 mg. respectively. From these results it would seem that the appearance of dental caries under these conditions is not due to the inability of the animal to

maintain calcium and phosphorus balance, since the greatest retention of these two elements was found in the rats which showed the greatest incidence of caries.

Group 'D.' The diet of this group was identical to the diet fed group 'C' except that 1 per cent of the weight of the diet in the form of washed, ignited sea sand was added to and mixed through the diet. The mesh of the sand was determined by, first, discarding all that portion of the sand that did not pass a 40-mesh standard sieve, and second, by then discarding all that portion that did pass a 60-mesh standard sieve.

Group 'E.' The diet of this group was identical to the diet fed group 'C' except that 5 per cent of the weight of the diet in the form of washed ignited sea sand was added to and mixed through the diet.

Twelve rats, 25 to 30 days old, from our own rat colony were fed this diet for 100 days, then chloroformed and examined for dental caries but no caries was found.

Group 'F.' The diet of this group was identical to the diet fed group 'C' except that 10 per cent of the weight of the diet in the form of washed ignited sea sand was added to and mixed through the diet.

Twelve rats, from 25 to 30 days old, from our own rat colony were fed this diet for 100 days, then chloroformed and examined for dental caries. It was found that three of these twelve rats (25 per cent) had one or more carious molar teeth.

DISCUSSION

Since the occurrence of caries, or its absence, was determined by feeding precisely the same diet in the uncooked state, or after it had been softened by cooking, the occurrence of the caries is not attributable to chemical differences in the two instances.

Furthermore, since caries could not be produced or prevented by feeding the same material in a coarse state or after it had been reduced to a fine powder, it is again clear that the caries we produced could not be directly related to the chemical constitution of the diet.

It should also be pointed out that the rats that developed caries retained more calcium and phosphorus than the rats that avoided caries.

It seems clear that the physical form of the diet was the predisposing factor of the occurrence of the caries. Since the particles of coarse cornmeal are exceedingly hard, it is conceivable that the animals fractured the enamel of their teeth as a result of chewing this material and that this initiated a destructive process.

It is, however, not clear how such a destructive process would take place, whether by digestion, the result of bacterial invasion, or failure of repair.

Another possible explanation appears to be worth mentioning, namely, that the particles of corn became wedged between opposing teeth, and that condition initiated any one of the three processes mentioned above.

We have however at the present no satisfactory explanation for the occurrence of the caries. We have nothing to offer other than the outstanding fact that coarse hard particles of cornmeal in the diet regularly caused the teeth of rats that ate this diet for 3 or 4 months to become carious to a high degree.

CONCLUSION

Diets adequate for growth, weight, and reproduction according to accepted standards, containing coarse cornmeal produced dental caries in 66 per cent of albino rats in 100 days and in 100 per cent of rats in 125 days.

The incidence of caries was related to the physical form of the food (cornmeal) and not to the calcium or phosphorus values of the diet.

If the coarse cornmeal were made soft by cooking or ground fine enough to pass a 60-mesh sieve no caries was produced. Vitamin D did not prevent the occurrence of the caries.

We wish to express our appreciation of the advice given us by Dr. L. H. Newburgh during this test.

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ANALYSES OF MEATS

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During the course of an investigation of the metabolism of two men who received an exclusive meat diet for 1 year, a series of determinations were made on the various cuts of meats which duplicated those consumed by the two men. A résumé of the observations of their metabolism was reported by McClellan and DuBois ('30), McClellan, Rupp and Toscani ('30) and McClellan, Spencer and Falk ('31). Sherman ('32) gives a detailed analysis of the edible organic nutrients, in which he differentiates between various cuts of the same foodstuff. In our investigation no attempt was made to sort the various cuts of beef muscle, the primary object being to determine the actual intake by the patients of the various organic and ash constituents. In this study the only source of carbohydrate was contained in the meat consumed by the subjects. The published tables (Sherman, '32) omit carbohydrate in their analyses, as the amount of carbohydrate contained in a portion of meat in a mixed diet is not significant.

This paper contains the values for protein, fat, carbohydrate, calcium and phosphorus as reported by McClellan, Rupp and Toscani ('30). The purpose of this report is to present a complete table on the meats analyzed including the total ash and water content.

PREPARATION OF MATERIAL

During the first 3 months the meat used was obtained from animals slaughtered the preceding day. After the third

month, ordinary refrigerated meats were used. Meals which duplicated as closely as possible those eaten by the men were sent to the laboratory daily in enameled pails and preserved on ice for the length of a period, which was usually from 7 to 10 days. The meat was then sorted, weighed, ground in a meat chopper, and dried on a steam bath in porcelain evaporating dishes. When dry, the specimens were weighed, ground and preserved in Mason jars for subsequent analysis. An aliquot portion of the stored sample was finely ground in a porcelain mortar, quartered and samples taken for duplicate analysis.

METHODS

Nitrogen

Duplicate samples of 0.5 gm. of the dried material were digested with 20 cc. of an acid mixture¹ in 500-cc. Kjeldahl flasks. The flasks were heated slowly until carbonization was complete; then the digestion was allowed to proceed at a more rapid rate until no particles of carbon were visible. The regular Kjeldahl procedure was followed from this point. The values for protein were obtained by multiplying the nitrogen in grams by 6.25.

Fat

Duplicate analyses were made on 5.0-gm. portions of the dried meats. The fat was extracted with anhydrous ether overnight, using the Soxhlet apparatus. The ether was recovered by distillation on a warm water bath. The fat residue in the Soxhlet flask was then extracted with redistilled petroleum ether, using the fraction which distilled over between 40° and 60° C. The fat containing ether was filtered through non-absorbent cotton into a Petri dish. The flask and funnel were washed several times with small quantities of petroleum

¹ The acid mixture consists of the following reagents:

5 per cent copper sulphate	50 cc.
85 per cent phosphoric acid	300 cc.
Concentrated sulphuric acid	100 cc.

ether to insure maximum recovery. The Petri dishes were then placed on a warm surface to permit the evaporation of the ether. When most of the ether had evaporated, the dishes were placed on a hot surface to remove the last traces of the solvent. After cooling, the extracted fat was weighed.

Carbohydrate

Hydrolyzable fraction. For this determination 0.5 gm. of the sample of dried meat was ground in a mixture of white sand and 2.2 per cent hydrochloric acid and made up to 50 cc. with the 2.2 per cent acid in a potato tube. The mixture was hydrolyzed for 5 hours in a boiling water bath. The hydrolyzed sample was neutralized with 50 per cent sodium hydroxide using litmus paper as the indicator. Four grams of trichloroacetic acid were added and the mixture was allowed to stand for 1 hour and then made up to 100 cc. with distilled water. The material was filtered, made up to volume and an aliquot portion taken for analysis. Determinations of the reagent blanks were made at the same time. The hydrolyzable fraction of the carbohydrate was determined by the procedure of Hagedorn and Jensen ('23). The hydrolyzed aliquot sample was made alkaline with 0.1 N sodium hydroxide using phenol red as indicator. Two cubic centimeters of 0.005 N potassium ferricyanide were added and the mixture boiled for 15 minutes in a boiling water bath. The tubes were allowed to cool to room temperature and 3 cc. of iodide mixture,² 2 cc. of 3 per cent acetic acid and 5 drops of starch were added. The solution was titrated against 0.005 N thio-sulphate, using a microburette graduated in 0.01 cc. As the usual starch solution was not as sensitive as desired, starch prepared in the following way gave delicate end points. One gram of soluble starch was ground to a paste with a small

² The iodide mixture consists of the following reagents:

Zinc sulphate	10 gm.
Potassium iodide	5 gm.
Sodium chloride	50 gm.
Made up in 200 cc of distilled water	

quantity of saturated sodium chloride solution. The pasty mass was made up to 100 cc. with hot saturated salt solution. This salt solution of starch, when well stoppered, kept for a long period of time.

Lactic acid. One gram of the dried meat was ground in sand and 4 per cent trichloroacetic acid, transferred to a 100-cc. measuring cylinder, made up to the mark with the 4 per cent acid and allowed to stand for 1 hour. The mixture was filtered into a 200-cc. stoppered measuring cylinder. Ninety cubic centimeters of the filtrate were neutralized with 50 per cent sodium hydroxide using brom-cresol-green as indicator. To the neutralized filtrate 40 cc. of 20 per cent copper sulphate and 40 cc. of a 10 per cent suspension of calcium hydroxide were added and made up to the mark with distilled water and shaken. The mixture was allowed to stand for 1 hour and then filtered through a dry paper. Twenty cubic centimeters of the filtrate were used for each determination. The procedure of Friedemann, Cotonio and Shaffer ('27) was followed from this stage.

Total carbohydrate. This was obtained by adding the values for the lactic acid content and the hydrolyzable fraction. It affords a fairly accurate measure of the total carbohydrate content of tissue. A small carbohydrate fraction, which is not susceptible to complete hydrolysis, is left undetermined by this method, but it is probably fairly constant under the conditions of the experiment and not significant in amount.

Calcium

Calcium was determined by McCrudden's method ('11, '12) on 10.0 gm. of the dried material after ashing.

Phosphorus

Phosphorus was estimated on 1.0-gm. portions of the dried samples by the volumetric molybdate method (Official and tentative methods of analysis of the Association of Official Agricultural Chemists, '25) after digestion with nitric and sulphuric acids.

Ash

The total ash was determined by ashing 10.0-gm. samples of the dried meats in porcelain crucibles. Samples that ashed with difficulty were treated with a few drops of nitric acid. Specimens that did not ash satisfactorily with the latter treatment were ashed by passing a slow stream of oxygen over the hot ash.

Water

The difference between the sum of the amounts of protein, fat, carbohydrate and total ash, and 100 was considered to be the amount of water present in 100 gm. of the fresh meat.

DISCUSSION

The results of the analyses which were made are presented in the table. Only the maximum, minimum and average values are included. There was close agreement between the values for protein obtained in this series and those reported in the standard tables.

The amount of fat in any cut of meat is a variable factor. Even in beef muscle which was trimmed as carefully as possible to remove all excess fat, variations in the fat content occurred between 2.9 and 7.6 per cent. This was due to differences in the amount of fat lying between the strands and fibers of the muscle which could not be removed by trimming. The amount of fat of this nature is dependent on the food with which the animal was fattened. In beef tongue, the variability in its fat content depended largely on the section of tongue used for the analysis. As these samples of meat were duplicates of those served in diets, sometimes there was a larger amount from the posterior portion of the tongue which was the section containing the greatest amount of fat and sometimes more from the anterior, lean portion.

In the standard food tables, the amount of carbohydrate in meat is entirely neglected and no values are given except for liver. In the studies for which these analyses were made, the extremely low carbohydrate intake made it important to

TABLE 1
Analysis of meats. Grams per 100 gm moist weight

NATURE OF MEAT	NUMBER OF SAMPLES	PROTEIN			FAT		Aver.	CARBOHYDRATE			CALCIUM		
		Max.	Min.	Aver.	Max.	Min.		Max.	Min.	Aver.	Max.	Min.	Aver.
Beef muscle, well trimmed	24	21.80	18.15	20.19	7.60	2.88	5.60	1.49	1.35	1.36	0.0152	0.0072	0.0144
Beef muscle, not trimmed	10	22.50	17.75	19.85	20.60	7.90	13.13	1.38	1.12	1.32	0.0169	0.0077	0.0119
Beef tongue	19	26.00	16.90	19.85	34.60	8.00	23.60	1.53	0.72	1.09	0.0150	0.0063	0.0081
Beef liver	14	24.30	15.30	20.20	8.55	4.20	6.34	3.47	1.70	2.52	0.0120	0.0056	0.0077
Beef kidney	2	15.40	13.90	14.65	2.50	2.40	2.45	0.40	0.40	0.40	0.0070	0.0070	0.0070
Beef brain	1			10.50			9.00			1.08			0.0077
Lamb muscle	4	16.00	13.95	15.14	33.60	22.90	27.00	0.76	0.54	0.69	0.0327	0.0109	0.0210
Veal muscle	3	18.80	17.20	17.80	17.20	13.50	15.96	1.62	1.38	1.50	0.0155	0.0134	0.0145

NATURE OF MEAT	NUMBER OF SAMPLES	PHOSPHORUS			ASH			WATER		
		Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Beef muscle, well trimmed	24	0.236	0.204	0.220	1.38	0.89	1.06	74.9	69.8	71.9
Beef muscle, not trimmed	10	0.260	0.194	0.222	1.23	0.85	1.01	69.9	54.4	65.7
Beef tongue	19	0.260	0.160	0.199	1.18	0.64	0.89	68.0	42.9	54.5
Beef liver	14	0.480	0.310	0.420	1.91	1.35	1.70	75.5	65.8	69.2
Beef kidney	2	0.252	0.252	0.252	1.10	1.06	1.08	82.1	80.7	81.4
Beef brain	1			0.380			1.61			77.8
Lamb muscle	4	0.189	0.170	0.180	0.99	0.77	0.85	59.7	49.9	56.4
Veal muscle	3	0.261	0.191	0.223	1.10	0.86	0.96	65.5	62.8	63.8

take into account even small amounts of carbohydrates. In order to include all material which might exert any anti-ketogenic effect, the total carbohydrates were taken as the sum of hydrolyzable carbohydrate and lactic acid as determined in the analyses. On the basis of this computation it was found that the men were taking 8 to 12 gm. of carbohydrate per day in their diets.

The determinations of minerals such as calcium and phosphorus and the total ash are of importance and emphasize particularly the low calcium content in meat. The higher values for phosphorus in liver and kidney as compared with the amounts in other cuts are well known.

The values for water only stress again the large amount of this constituent in many of the foods in the normal diet and the absolute necessity of considering the water in food in any studies regarding the water balance.

SUMMARY

1. Analyses are reported for seventy-seven samples of meat. They include beef muscle, tongue, liver, kidney and brain. A few samples of lamb and veal muscle were analyzed. The significant features of these analyses have been considered in the previous discussion.

2. In order to have a good check on the values of the various foodstuffs consumed when meat makes up a considerable portion of the diet to be studied, it is important to make analyses of samples of meat which duplicate as closely as possible the meat eaten.

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A NEW TECHNIC FOR THE MEASUREMENT OF
AVERAGE SKIN TEMPERATURE OVER SUR-
FACES OF THE BODY AND THE CHANGES
OF SKIN TEMPERATURE
DURING EXERCISE ¹

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SIX FIGURES

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The earliest measurements of skin temperature were made by Davy in 1814, by holding the bulb of a thermometer against the skin. Although obviously considerable errors are introduced, this method is still employed and perhaps is of sufficient accuracy in cases where interest centers in changes in the temperature of a particular area and where the absolute value of the temperature is immaterial. The introduction of thermocouples brought a great improvement in accuracy and many measurements of skin temperature at different parts of the body have been made by this method by Benedict, Miles and Johnson, Benedict and Parmenter, Talbot, Cobet and many others. A review of the literature is given by Cobet ('26). Their apparatus is fully described in the papers referred to. Aldrich ('32) pointed out that the type of construction of the thermocouples used in the majority of these experiments introduced a fundamental error. One or more junctions of the thermocouple were pressed against the skin or clothes by an insulating holder. Behind the junctions was a pad of cotton wool or other heat insulating material. The temperature of the skin is such that there is an equilibrium

¹ This study was supported by a grant from the Rockefeller Foundation.

between the heat reaching it from the interior and that lost by convection, radiation and evaporation. The application of a thermocouple of the type described necessarily interferes with the heat loss from the area with which it is in contact, and there is a consequent rise in temperature of an undetermined amount. It is thus essential that the portion of the thermocouple that is in contact with the skin should have as small a heat capacity as possible and have a minimum effect upon loss of heat. Thermocouples of fine wire without backing of insulating material other than a fine silk thread have been used by Aldrich and fulfill the conditions well. The thermocouples used by McClure and Sauer ('15) also were satisfactory in this respect, but this source of error seems to have been overlooked in more recent work.

These experiments have revealed that there are very considerable differences, amounting to as much as 10°C . between different parts of the surface of the body when it is in a cold environment. When studies are being made of the changes in skin temperature from individual to individual and in the same individual under different conditions, it is the average skin temperature over large areas of surface or over the whole body that is of significance. Also it is recognized that the average skin temperature over the body determines the heat loss to a great extent and a knowledge of this would be a valuable index to heat loss and metabolism. The great range of temperature in the different parts of the skin surface renders the estimation of such an average skin temperature by the use of thermocouples very difficult. The method to be described has been designed to give directly an average of the temperature over an extended area, such as the trunk, with the least disturbance of the conditions existing before the measurement is made. It is adapted to give continuous records of the temperature in conditions such as in the measurement of the skin temperature under clothing, during exercise and sleep, in which the thermocouple methods are inapplicable. Where interest centers in local changes of temperature of small areas, as in examination of the sympathetic

vasoconstrictor activity (Morton and Scott, '30), it has no superiority over other methods.

For the thermocouple, a resistance thermometer is substituted. This type of thermometer was used by G. N. Stewart (1891) for measurements of skin temperature. The principle has been in use at the Russell Sage Institute and thermometers have been described recently by Soderstrom ('33). The thermometers here described differ greatly, however, from those employed previously.

Nickel has a large temperature coefficient of resistance of about 0.4 per cent per degree centigrade. It was found possible to fix a continuous length of fine nickel wire, of no. 40 gauge, enameled and cotton covered, to cloth by sewing. The nickel wire was placed in the lower spool of a sewing machine while ordinary thread was used above. When proper adjustment of the tension of the thread was attained, the nickel wire was held to one side of the cloth without serious kinking which would tend to cause breakage of the wire. In this way wire was fixed in lines about $\frac{1}{2}$ inch apart up and down a piece of cloth shaped to fit as a jacket around the trunk of the subject. The ends of the wire were anchored securely by sewing to the cloth and connected to flexible leads. In order that the wire might be held closely in contact with the skin the jacket was fastened at the back by adjustable elastic tapes. Adjustable shoulder straps made the vest adaptable to various subjects. In all there were some 40 feet of wire in the jacket and the resistance was of the order of 200 ohms. Its resistance therefore changed by about 1 ohm for every degree centigrade.

Similar 'jackets' were made using both medium weight cloth, such as is used for nurses' uniforms, and single weight gauze; the latter being designed to give least interference with the evaporation and loss of heat from the bare skin. A stocking similarly constructed, to measure the skin temperature over the leg has also been used, and the method of construction can be used to make a thermometer adapted to almost any area desired.

To record the changes in resistance, and therefore of temperature, the usual arrangement of the Wheatstone network was used, shown in the diagram (fig. 1). Coils were wound of constantan wire, which does not change its resistance with temperature, and a special box was made up, but any form of resistance bridge available might be used. The two 'ratio arms' are equal, of 300 ohms each. The other two arms of the bridge that are being compared are alternatively, by the throw of a double pole, double throw, switch, either the jacket

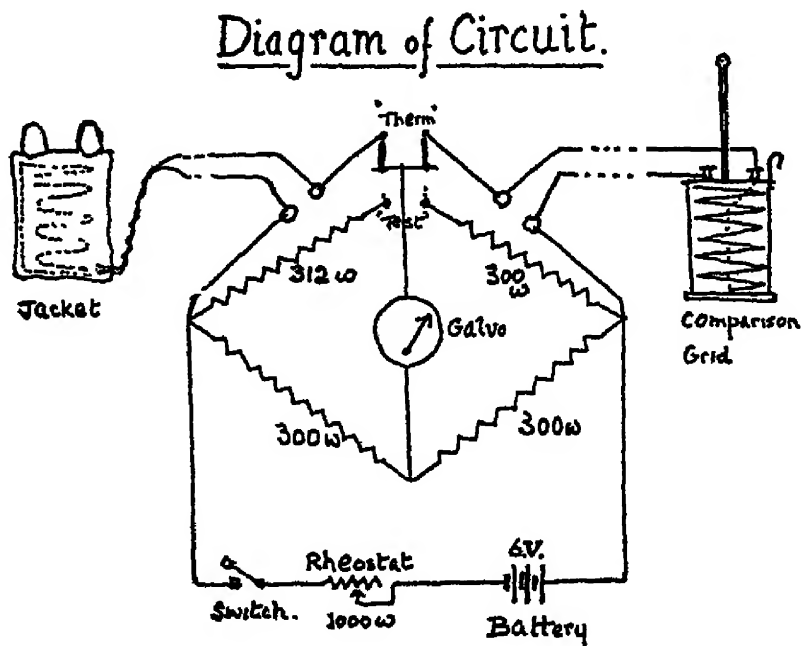


Figure 1

resistance and a comparison resistance, or with the switch in the 'test' position, two fixed constantan coils that differ in resistance by a known amount, say 12 ohms. The comparison resistance consists in the first place of a constantan coil of resistance such that it is equal to that of the jacket at some standard temperature, say 24°C. With the jacket at that temperature there is then no deflection of the galvanometer. At any other temperature the nickel wire in the jacket has a different resistance, the balance of the bridge is disturbed, and the deflection of the galvanometer is very closely proportional to the change in temperature of the jacket.

In use the switch is first put to the 'test' position and a deflection results which is equivalent always to the deflection that would be produced by a certain difference of temperature of the jacket above the zero temperature (the 24°C. mentioned). The value of this difference of temperature was determined by a calibration experiment in which the jacket in a metal container was immersed in water baths of known temperatures. For instance, it was found that 10°C. difference of temperature in the water baths produced a change in the deflection of, say, fifteen divisions of the scale of the galvanometer, while in the 'test' position the deflection was twelve divisions. The test deflection therefore corresponded to $12/15 \times 10 = 8^\circ\text{C}$. If then in any subsequent experiment the deflection corresponding to the temperature of the jacket is 'x' divisions, while the test deflection is 't' divisions, the jacket must have been at a temperature $x/t \times 8^\circ\text{C}$. above the temperature level. The latter temperature is found from the same calibration experiment, since from the deflections at two different temperatures it is easily deduced at what temperature the deflection would be zero. Thus an equation is found by which deflection may be turned into temperature. In the case cited above it would be:

$$T = 24.0^\circ + x/s \times 8^\circ\text{C}.$$

The advantage of this system of a 'test' deflection is that it renders the method independent of the particular galvanometer used and of the current in the bridge. If the sensitivity of the galvanometer or the voltage of the battery supplying the current change in the course of an experiment, as long as test deflections are taken before and after the change, no new calibration is needed.

It is obvious that by adjustment of the current in the bridge so that the test deflection is some standard value the temperatures may be read off the scale directly, as for instance, if the test deflection 's' was made to be always eight divisions of the scale in the example above. Each division would then represent 1 degree. If 's' were made four divi-

sions, each division would be equivalent to 2 degrees. The sensitivity can thus be conveniently adjusted to any approximate value. Resistance thermometry has the great advantage over the use of thermocouples in that the sensitivity can be increased merely by increase in the current through the bridge supplied by the battery. Thermocouple deflections can be increased only by the use of a more sensitive galvanometer.

The galvanometer used in the majority of experiments was of the 'thread recorder' type made by the Cambridge and Paul Instrument Company. This records the deflection upon a chart on a moving drum at intervals of 1 minute. It is fitted with a switching mechanism so that records of two different currents may be, if desired, recorded simultaneously. Any other type of galvanometer of sufficient sensitivity, recording or otherwise, may, of course, be used. With the particular arrangement used a sensitivity of about 0.02°C. was obtained when a current of about 0.01 ampere flowed in the bridge. In view of the physiological variations no advantage would be gained by increasing the sensitivity. It may easily be shown that the heating effect of a current of this magnitude in the wire of the jacket introduces a negligible error in the determination of the skin temperature.

For the estimation of heat loss from the body, it is not the absolute level of skin temperature but rather the 'excess temperature' between the skin temperature and the surroundings that determines the heat loss. It has often been pointed out that the heat loss by radiation and convection is proportional to the excess temperature. In order that the excess temperature might be recorded directly, a grid of the nickel wire of the same length as in the jacket was sewn in a piece of the same material and fixed around a wooden frame. The wire lies on the inside of the cube thus formed, its two ends being brought out to leads connected to the bridge in place of the fixed constantan comparison resistance. Adjustment of the length of nickel wire in this comparison grid, so constructed, was carefully made until when jacket and comparison grid were at the same temperature there was no deflection

of the galvanometer. The subsequent deflections then indicated directly the difference of temperature between the skin of the subject and the temperature of the air at the place where the grid is placed. When the subject is lying in bed, the latter is hung by the hooks shown to the rail of the bed. Since the construction of the comparison grid is similar to that of the jacket, fluctuations in the room temperature will affect the two to the same extent and with about the same lag in time. Thus a record of the excess temperature may be made over a long period, irrespective of fluctuations of room temperature, as in the case of the skin temperature of a sleeping



Figure 2

subject through the night. The comparison grid is shown to the right hand side of the photograph of the apparatus (fig. 2).

It was found that from 10 to 15 minutes was amply sufficient for the temperature of the jacket to have reached the maximum temperature, i.e., that of the skin. After that point the temperature recorded would remain constant to well within 0.1°C . provided there were no physiological changes in the subject. Draughts of air, the proximity of any radiating body such as a person or a strong light, any sudden move-

ments of the patient, or the onset of sweating show themselves immediately. An example of the constancy of the temperature is shown in figure 3, from an actual record of the galvanometer.

Studies are being made of the level of skin temperature over the chest in normal and febrile subjects of different sizes and ages under basal conditions. For this purpose it is desirable that the area of skin measured should not extend to the part of the back that is in contact with the bed, as movements here would alter the heat loss of this area and thus the recorded temperatures. Accordingly the 'jackets' are here simply rectangular pieces of gauze sewn with wire as described. The sizes are such that when tied around the trunk of the subject the area covered by the nickel wire ends just posterior to the line from shoulders to hips.

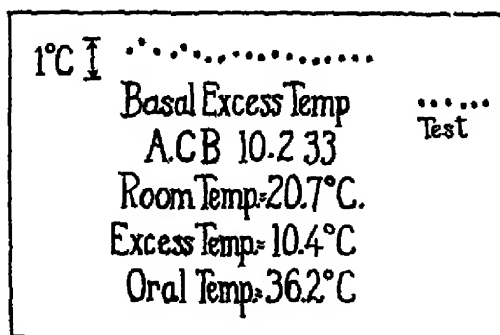


Figure 3

By the aid of this technic studies are being made of the mechanism of heat loss and of the heat regulating mechanism of the body. These are not yet complete, but as an example of the use of the method some studies of the skin temperature during and after muscular exercise are here reported.

Benedict ('25) and Benedict and Parmenter ('28) studied the effect upon the skin temperature at a number of points of the body of exercise such as outdoor walking, running and work upon a treadmill in the laboratory. They used thermocouples and had no means of recording the skin temperatures during the performance of the muscular work. Readings taken before and after the exercise showed some inconsistencies but they concluded that in general "there was a distinct drop in average peripheral temperature as a result

of muscular activity for five minutes, in spite of the fact that the metabolism is greatly increased and a greater heat loss would be expected." They suggest that this might be due to a vasoconstriction of the peripheral vessels to transport blood from the periphery to the muscles. Talbot ('31) came to the same conclusion with children as subjects. A contrary rise of temperature was sometimes found in the case of the hands and cheek, although the temperature of the forehead always fell markedly.

The technic described offered a means of observing the changes of skin temperature during the exercise. The work was performed and measured upon a magnetically braked bicycle ergometer. The subject wearing a gauze resistance jacket over the bare chest sat at rest in the saddle for 10 or more minutes until the skin temperature was at a constant level. The rectal temperature was taken during this period. He then began pedaling, at the point marked A on the records, against various loads and at measured rates, for a period of 10 minutes, ceasing at the point C on the records. Except for an interruption to take the rectal temperature after the exercise, he then remained at rest on the bicycle until the skin temperature was again normal.

Some typical records for increasing amounts of work are shown in figure 4. These are actual charts made by the recording galvanometer, the dots being merely emphasized and joined by a line for sake of clearness. There are of course inconsistencies in individual cases but the general trend of the curves may be described as follows.

Immediately upon the commencement of the work, at the point marked A on the charts, a rise in skin temperature was evident. This amounted to 0.2° or 0.3°C . and occurred in less than a minute after the start of the exercise. In some cases it was observed, though not seen upon the record, since the galvanometer marks only the deflection at the end of each minute. The initial rise was followed by a period in which the temperature remained practically constant until after some 6 to 8 minutes there began usually a fall in skin

temperature at a quite definite point marked B in the records. The work ceased at the point C, upon which there was in most cases immediate acceleration of the fall of temperature. After some 5 to 10 minutes the lowest temperature was reached, considerably below that which prevailed before the exercise. The amount of this total fall is seen to increase

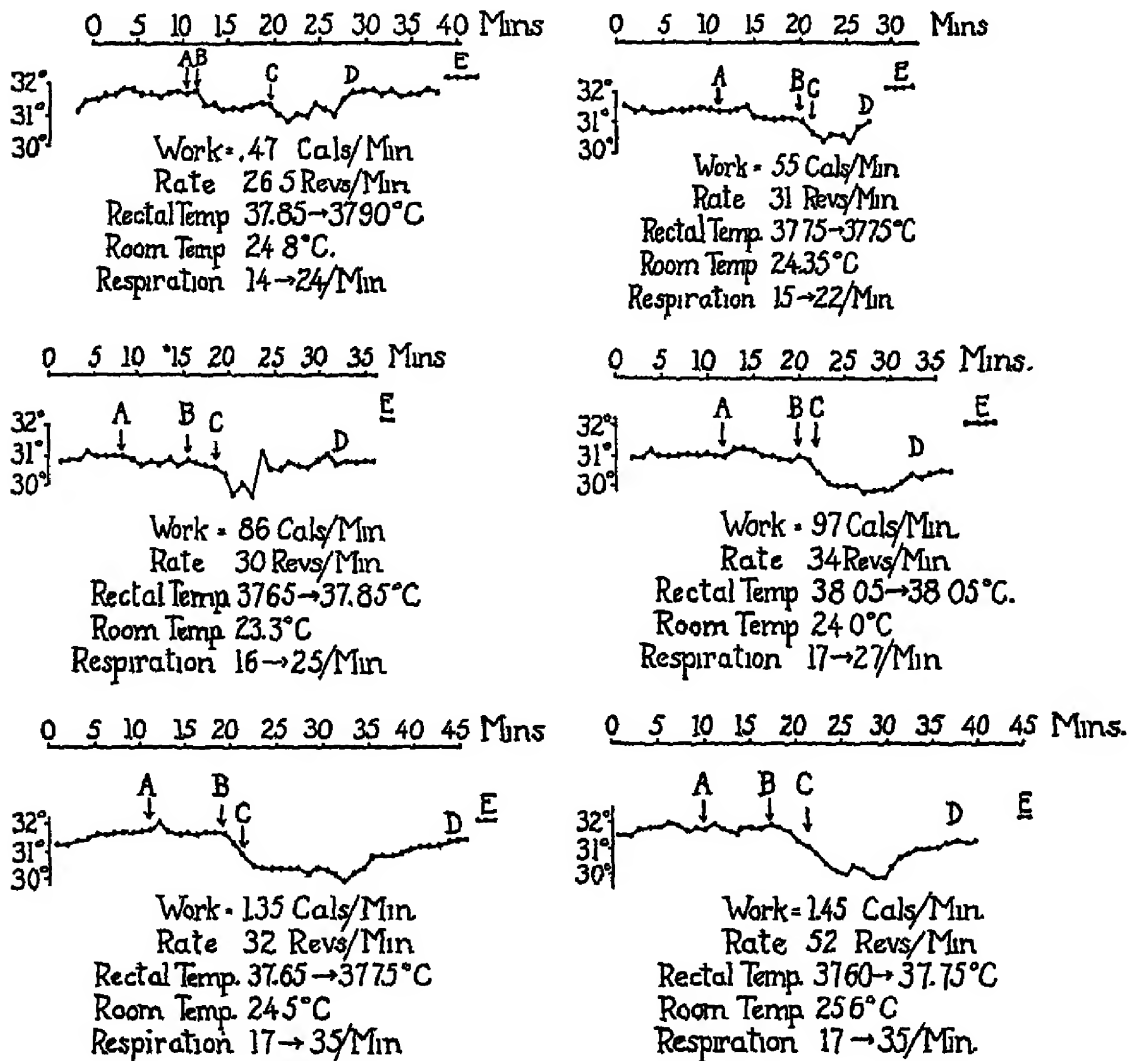


Figure 4

with the severity of the exercise, from 0.08°C. for the lightest load to nearly 2°C. for the heaviest. A subjective sensation of sweating, apparent first upon the forehead, coincided with the point B, and there was a feeling of cold on the skin after the cessation of work at C. The temperature then gradually rose until some 15 to 20 minutes after the end of exercise the temperature had reached constancy at a level equal to or

slightly lower than the original level before exercise. The points marked E on the charts are the 'test deflections' explained earlier in the paper. As is well known in the physiology of muscular exercise, there was in general a rise of rectal temperature which was greater the greater the work done. For the heaviest loads it amounted to 0.2° . The respiration rate increased proportionally to the work done.

The natural interpretation of these recorded changes in skin temperature is that the initial rise is due to the increased circulation upon starting the work, which brings up more heat to the surface of the body. A similar rise in the skin temperature has been recorded, in the case of subjects lying quietly on a bed, following the smallest movement or even tension in the muscles. The fall of temperature starting at B can evidently be attributed to an increase in the evaporation of water from the skin, produced by a stimulated activity of the sweat glands starting at this point. Thus when the exercise ceases at C, this evaporation continues to cool the skin, and since the circulation quickly returns to normal there is no compensating increase in heat brought to the skin to slow the fall of temperature. The total drop of temperature produced by the evaporation would naturally depend upon the total amount of sweat to be evaporated, and thus increased with the severity of the exercise. Finally sweating has ceased and as soon as the remaining water has been evaporated and the skin dried the temperature returns to normal. The tendency to stay at a slightly lower level after the exercise from that preceding it may be evidence of a compensating decrease in the circulation after the period of extra load upon the heart or may merely indicate that drying is not complete for a long time.

Confirmation of the view that the decrease in skin temperature during exercise is due to sweating is furnished by some experiments in which the gauze resistance jacket was replaced by one of 'uniform cloth.' The effect of this is to allow the extra heat during exercise to accumulate at the skin and to delay the effects of evaporation. A typical curve is shown

in figure 5. The phases that have been described are now much more clearly demarked. The initial rise of temperature is much greater, amounting to $1.5^{\circ}\text{C}.$; the onset of sweating at B is plainly seen and the increase in the slope at C when work ceases. The final drying of the skin and rise of temperature to the original level is, as would be expected, much slower.

It was thought of interest to compare the changes in skin temperature on the leg of the subject when riding the bicycle, as here in addition to the increased circulation there is considerable work done by the underlying muscles. A gauze

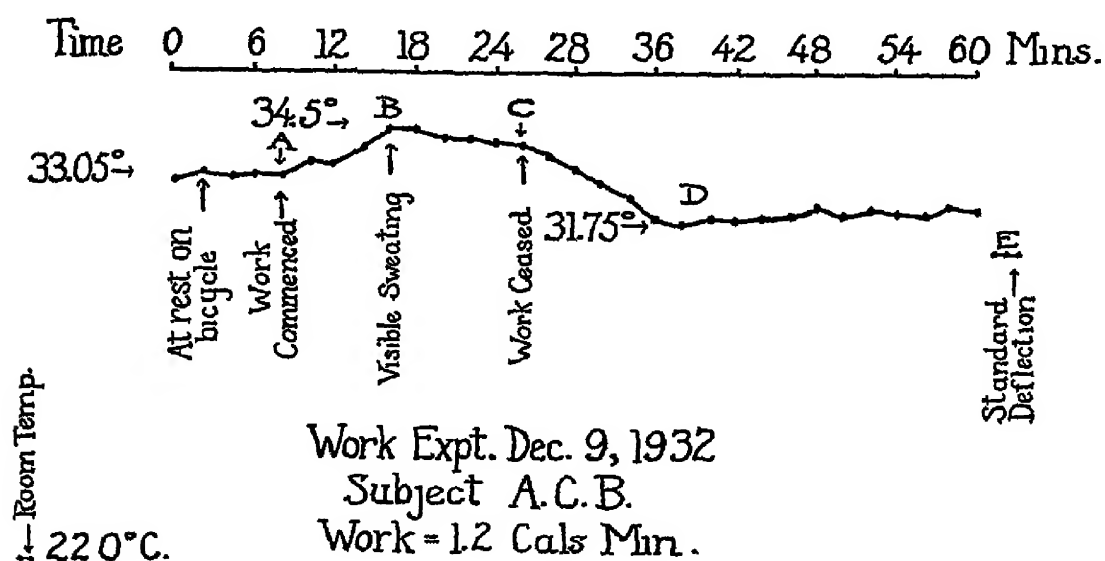


Figure 5

legging was fitted so that the resistance wire lay in contact with the area over the muscles in the back of the leg. The results are shown in figure 6. In (A) the procedure was as before. The subject sat with the feet on the pedals but motionless until the work commenced at the point A. In this case there was no evidence of an initial rise but an immediate drop of temperature. After 4 or 5 minutes the temperature became constant at a lower level and showed some tendency to rise. At B a new fall began, presumably due to sweating. When work ceased at C a sudden temporary rise in temperature was seen, then a final steady rise to the original temperature as with the temperature of the trunk. It seemed

possible that the differences in behavior from the latter were due to the increase in the ventilation of the skin of the leg when pedaling began. To test this the experiment shown in figure 6 (B) was made. Here after sitting at rest for several minutes, pedaling was started at A with no brake load on the wheel, so that very little external work was being done. The immediate drop to a lower level of temperature

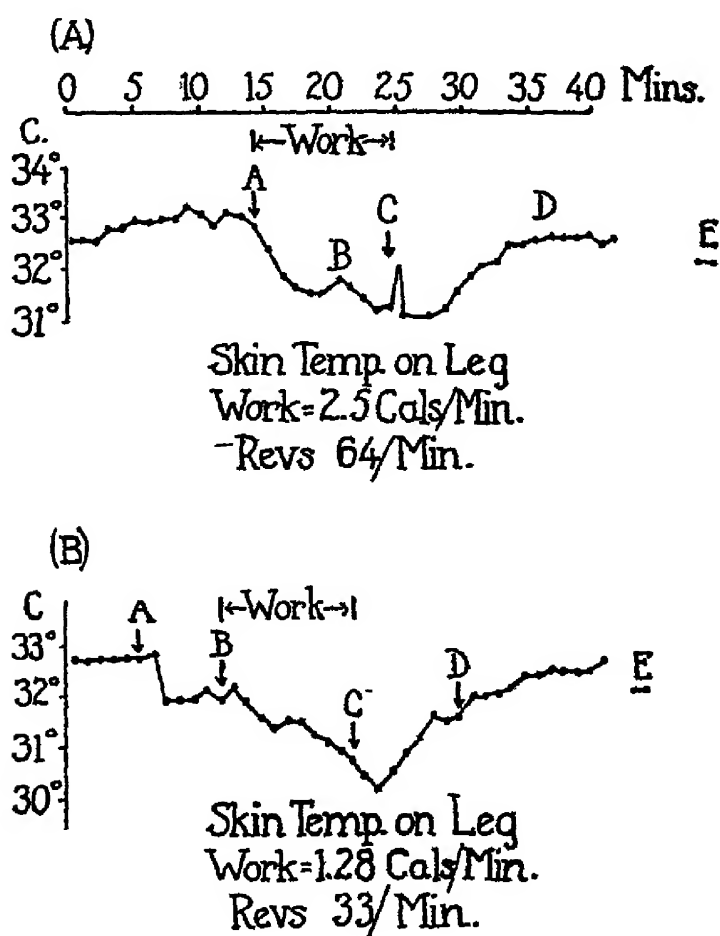


Figure 6

shows how great was the effect of the motion of the air. At B the magnetic brake was switched on, and there was now some indication of an initial rise, followed by the normal fall. At C the load was removed but free pedaling continued until the temperature had reached constancy at D, when the pedaling ceased. There then occurred a new rise of temperature until the original resting level was reached. When the effect of the motion in creating a cooling wind on the legs is taken

into account, the changes in skin temperature on the leg closely parallel those of the trunk.

A great many more experiments would be necessary in order that the onset of sweating might be correlated with other physical factors. It certainly appears from these experiments that the absolute level of the temperature of the skin does not play a dominating part in controlling the onset of sweating in muscular exercise. If it did so, we would expect to find that the maximum temperature reached by the skin would be constant. On the contrary, the maximum temperatures and those at which sweating occurred (the points B on the diagrams) varied from 31°C. to 34.5°C., the latter being under the cloth jacket. There is no indication that in the cooler environment the onset of sweating is delayed. The stimulation of sweating must in the case of exercise be referred to some other factor than sensory stimulus from the skin—possibly to chemical changes in the blood. The correlation with the amount of work done is, however, not at all good.

SUMMARY AND CONCLUSIONS

A technic is described, adaptable for continuous recording, which gives directly the average skin temperature over an extended area such as that of the trunk, with very little disturbance of normal conditions. A considerable length of fine insulated nickel wire is fixed by sewing to the inside of a jacket of light gauze or cloth, so that it is held in close contact with the skin of the subject. Its resistance is balanced in a Wheatstone bridge against a coil of fixed resistance, or against a similarly constructed resistance of nickel wire which is kept at room temperature. Deflection of the galvanometer records in the first case the level of the skin temperature, or in the second case, the excess temperature between skin and room. The use of the method is illustrated by experiments on the skin temperature of a subject doing muscular work on a bicycle ergometer.

1. During muscular exercise there is an initial tendency for the skin temperature of the trunk and leg to rise. This rise may be obscured by the cooling effect of the wind caused by the motion.

2. The rise is quickly halted and followed by a fall of temperature, starting quite definitely at a sharp point, which corresponds to the subjective sensation of sweating. The evidence suggests that this fall is due to the cooling by evaporation of sweat, rather than to the shift of blood flow suggested by Benedict and Parmenter.

3. Upon cessation of exercise, the fall of skin temperature is accelerated, while evaporation continues and the extra heat is no longer brought up to the skin.

4. For some 10 to 15 minutes after the exercise the skin temperature is depressed up to $1\frac{1}{2}$ or 2°C . depending on the severity of the exercise, below the normal level.

5. As the skin dries out and sweating ceases, the original level is gradually approached.

6. The onset of sweating in muscular exercise is not controlled by the level of skin temperature reached, but must be referred to some other factor.

Grateful acknowledgment is made of the interest and encouragement of Prof J. R. Murlin, and of the technical assistance of Mr. Knight of the Orthopedic Shop of the Strong Memorial Hospital in the construction of the resistance thermometers.

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THE APPLICATION OF THE THEORY OF HEAT FLOW TO THE STUDY OF ENERGY METABOLISM

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FIVE FIGURES

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Since the days of Lavoisier (1777) the animal body has been recognized as a generator of heat. The development of respiration calorimetry has placed the knowledge of this generation of heat by metabolic processes upon a fairly exact quantitative basis. This generated heat eventually becomes heat lost from the body to its environment. Here 'direct calorimetry' has provided quantitative information as to the heat loss and its partition into the various modes of dissipation, namely, radiation and convection, conduction and evaporation of moisture. The transition processes by which heat generated within the body becomes heat lost from the surface have not, however, received much attention, and few attempts have been made to apply quantitative laws in this field. Notable among the attempts were the experiments and conclusions of Lefèvre in his work on 'Animal Heat' ('11). Yet there seems to be possible a considerable advance in clarity of thought, if in nothing else, by the study of this transfer of heat in the light of the laws so well known to apply to purely physical systems. If these laws are held to be of little application to the living animal body on the score that this is something more than a purely physical system yet the question of how much and of what nature this 'something more' may be, cannot be answered until the behavior of the

underlying purely physical system is understood. In that sense physiology must start with physics.

In the application of these laws that follow, a fundamental supposition is that the body is in a state of thermal equilibrium, that is, that heat production is balanced by heat loss so that the temperature of any given part of the system remains constant in time. It is probable that this assumption is never completely justified in the case of the human body. Changes in the heat production, due to muscular activity, ingestion of food and a great number of other factors, cannot be accurately and immediately compensated by corresponding changes in heat loss. The result is the well-known fluctuation of the average temperature of the body as a whole and of the distribution of temperature in its parts. The regulating mechanism possessed by the warm-blooded animals operates to prevent this normal fluctuation of temperature from exceeding limits that are quite narrow, so that over a long period of time heat loss is accurately equal to heat production. Were this assumption of thermal equilibrium not made, the mathematical analysis would become much more complex. Partial differential equations containing time as well as position as a variable would be involved. To a certain extent, the results in cases where thermal equilibrium is not attained may be deduced from the results of the simpler case. It must be remembered that the results here cited are applicable strictly only to the case where the simplifying assumption is justified.

The fundamental law of heat flow is that it does so spontaneously only from a place of higher to one of lower temperature. For any flow to take place there must be a 'gradient of temperature.' This law is, of course, intimately connected with the second law of thermodynamics and it may be taken as established that any apparent contradictions cannot stand upon examination. Further, the flow of heat increases as the gradient of temperature becomes greater. As an illustration of the importance of this law in the case of the human body, we might imagine that we were able to revive miracu-

lously the metabolic processes in a lifeless body that has cooled to the temperature of its surroundings. The heat once more generated by metabolism would be at first totally, later partially, used in raising the body temperature. Since initially there was no gradient of temperature no heat could flow to the surroundings. As, however, the body temperature rose, a temperature gradient of increasing magnitude would be established and an increasing heat loss to the surroundings. Finally, a steady state would be reached when the gradient was sufficient to give a heat flow to the surroundings that exactly balanced the heat generated in the body. There would then be no further rise of body temperature. (A simple calculation shows, however, that it would take at least 15 hours at the normal basal rate for the body temperature to reach to within one-tenth of the final temperature above the surroundings. This is for a body of 80 kg. weight.) This imaginary experiment is closely paralleled by what actually occurs during the waking of the hibernating animal.

The normal body temperature, about 37°C . in humans and a little higher in many mammals and birds, is thus maintained constantly at a higher level than that of the surroundings, which we may suppose to be from 20°C . to 25°C . in normal circumstances. This gradient is sufficient to dissipate all the heat generated by the metabolism. The heat generated in the deep interior of the body is flowing out to the surface, augmented on its way by the heat generated in the more peripheral tissues. This flow demands that there be a gradient of temperature from the interior to the surface of the body as well as a gradient from the surface through the clothing to the surrounding air. Thus, in surroundings at, say 24°C ., the average skin temperature over the surface of the clothed body, varying of course considerably in different parts of the body, is from 30° to 33°C ., while the internal body temperature is approximately 37°C . A considerable portion of the total drop of temperature from the body to its surroundings thus occurs within the body itself. We may usefully distinguish between the two portions of the total drop of tem-

perature, namely, the internal 'physiological' drop of temperature from the point in the interior of the body where the temperature is a maximum to a given point on the surface, and the external 'physical' drop of temperature from that point on the skin surface to the surrounding air.

This distinction is a natural and a very convenient one. Over the first of these temperature gradients, namely, the physiological internal gradient, man, in common with other warm blooded animals, has a considerable control by regulation of vasomotor activity; over the second, the external gradient, he has only the measure of control that he has learned to exercise by the wearing of clothes and by the conditioning of his environment. It would appear that lack of distinction has led to the occurrence of misleading statements in the literature of energy metabolism. It is reiterated, for instance, by many since Lefèvre made the statement, that the purely physical laws of heat loss have little if any application to the human body as shown by the fact that the heat loss is not found to be correlated with the difference of temperature between the body and its surroundings as these laws would predict (Hoesslin, 1888; Richet, 1889; DuBois, '16). Yet there can be no shadow of doubt that the purely physical laws are applicable to the loss of heat from the surface of the body, the skin, to the air across the external 'physical' gradient. It is in their extension to include the physiological gradient within the body that the physical laws require some modification—a modification which it is the aim of this paper to explain. It has also been argued that the skin temperature is not a reliable guide to the heat loss, since by a modification of the internal gradients within the tissues more heat might be lost without a change in the temperature of the surface (Barr and DuBois, '18). A realization of the purely physical nature of the external gradient of temperature from the skin to the environment shows that this statement is erroneous.

The external gradient. The external gradient will first be considered. The laws that govern the flow of heat from a hot body to its cooler surroundings have long been known. New-

ton summed up his researches on the subject by the well known 'Newton's law of cooling.' It makes no claim to be anything but an empirical law and as such still stands. It states that the rate of cooling, indicative of the rate of loss of heat, is directly proportional to the difference of temperature between the body and its surroundings. The heat loss occurs partly by radiation, partly by convection and conduction, and in the case of the animal body, partly by evaporation of water from the surface of the body. The laws that govern these individual processes are quite widely different. For radiation there is the well-known Stéfán's fourth power law, that the radiation is proportional to the fourth power of the absolute temperature. Its application to radiation from the human body has been shown by the experiments of Aldrich ('28). The laws of convection are complicated and not yet defined to cover all cases, those of conduction are well established. Newton's law is an empirical law applying to the particular combination of these modes of heat loss that are normally found in the case of a body cooling in air. The amount of evaporation occurring is dependent primarily upon the difference of vapor pressure of the moist surface—that of the blood and of the sweat in the sweat ducts—and the vapor pressure of water in the air. In the range of temperature in which we are interested the vapor pressure of water increases with the temperature in a way which, though not linear, is not far from it. The total result of all these factors is that we may apply Newton's law as of the best applicability with simplicity to the loss of heat from the skin. It is also accurately true that in conditions otherwise the same, the loss of heat per unit of time is proportional to the surface area of the hot body. Summarizing this in an equation, if H^1 is the rate of heat loss, say in kilogram calories per hour, T_s the temperature of the skin, A the surface area, and T_e that of the surroundings:

$$H^1 = C (T_s - T_e) \times A$$

or dividing throughout by the area, A , and using H for the Calories per square meter per hour—

$$H = C (T_s - T_e) \tag{A}$$

The constant 'C' is one that indicates how easily, under given conditions of ventilation, of clothing and of humidity, the heat flows from the skin to the surroundings. It may be called the 'coefficient of heat transfer' from the skin to the environment.

It is perhaps preferable to separate the heat lost by evaporation from that lost by other routes, since this mode may be considered to depend upon a different set of physiological variables from the loss by radiation and convection. We then rewrite equation (A) in the form—

$$H = C (T_s - T_e) + H_e \quad (B)$$

where H_e is the heat lost per unit area per unit time by the evaporation of water.

A simple physical experiment was made to verify that the linear law of heat loss expressed by (A) was valid in the range of temperatures met with in metabolism studies. The bulb of a catathermometer was wound with a coil of resistance wire so that by passing a known current through it the thermometer bulb could be held at a steady state of excess temperature over its surroundings. The coil was covered with a layer of cloth held round the bulb, so that the condition in the experiments to be described might be paralleled. The heat generated, known from the resistance of the coil and the current flowing from it, was in the steady state equal to the heat loss. This was plotted against the excess temperature (fig. 1). The temperatures of the bulb were from 30°C. to 40°C. and the surrounding temperatures from 20°C. to 30°C., the air being still. The reason for the experiment, which must have many times been performed, was to discover how the humidity affected the heat loss by convection and radiation from a hot body in still air.¹ The results show that its effect is negligible for humidities that are encountered in normal conditions. In estimating the measure of heat lost by radiation and convection by the excess temperature above the

¹Thanks are due to the Eastman Kodak Company of Rochester for the use of a constant temperature and humidity room in these experiments.

surrounding air we therefore use the dry bulb temperature, rather than the wet bulb temperature or some combination of the two such as the 'effective temperature' of Yaglou ('26) and his co-workers. The latter is of significance in the estimation of heat loss by evaporation as well as by other routes, while here we deal with the evaporation loss separately.

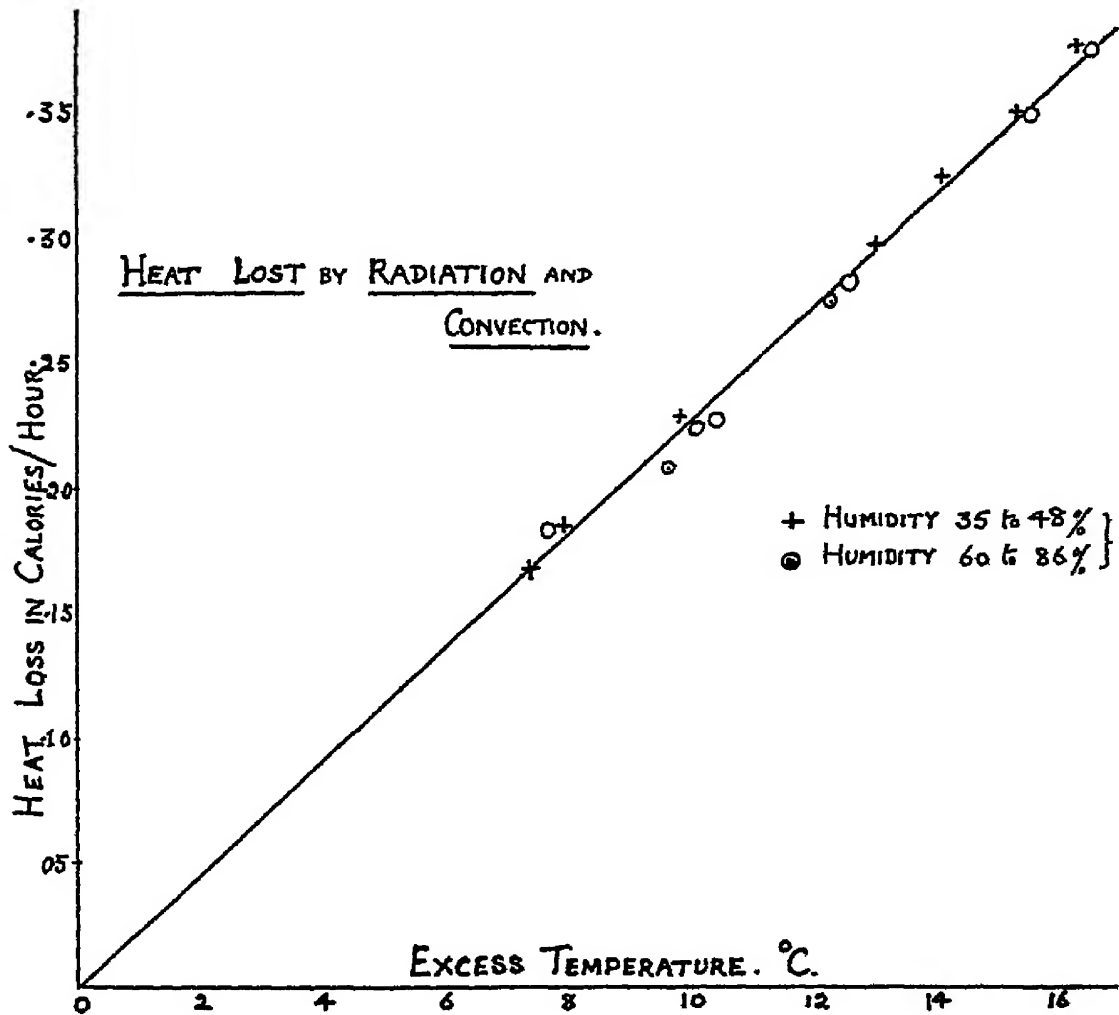


Figure 1

An estimate of the order of magnitude of the coefficient C in normal circumstance for the human body may be easily made. For adults H is about 30 kg.-Calories per square meter per hour. Assuming an average skin temperature under the clothing as $32^{\circ}\text{C}.$ in an environment of $24^{\circ}\text{C}.$, the excess temperature is of the order of $8^{\circ}\text{C}.$ Throwing these quantities into the fundamental units of small calories, square centimeters, and seconds, we find by substitution in equation (A)—

$$C = 1.05 \times 10^{-4} \text{ Cals./sq.cm./sec./}^{\circ}\text{C.}$$

The exact value of C , of course, depends upon the clothing worn. By estimating the surface area of the radiating bulb in the experiment described, the results of which are given in figure 1, a value of the same order results for the coefficient of heat transfer into still air.

Returning to consider equation B, it is a fortunate circumstance that in a variety of conditions and with a diversity of subjects the heat loss by evaporation, H_e , has been found to be a very constant fraction of the total heat loss, provided that there is no sweating. So constant is this fraction that

TABLE 1
W. Leet. Weight, 23.6 kg. Height, 128 cm. Age, 7 years

DATE	ROOM TEM- PERATURE	SKIN TEM- PERATURE	EXCESS TEM- PERATURE	RECTAL TEM- PERATURE	CIRCUL- ATION INDEX	METABOLISM CALS / SQ M / HR	REMARKS
1932							
12-21	25.5	33.5	8.0	36.7	2.5		
12-22	27.5	35.1	7.6	36.7	4.75	42.7	
12-23	26.65	36.1	9.45	37.2	(8.6)	45.2	Skin temperature probably in error
12-24	28.1	36.0	7.9	37.6	4.95	44.7	
12-25	27.2	35.85	8.65	38.2	3.7	51.9	
12-26	26.4	37.5	11.1	39.0	7.4	54.0	Rash appeared
12-27	27.2	37.7	10.5	38.4	15.0	45.2	Rash at maximum
12-28	27.5	36.9	9.4	38.6	5.5	54.1	Rash decreasing
12-29	26.6	34.65	8.05	38.2	2.3	53.1	Rash gone
12-30	27.0	35.9	8.9	38.7	3.2	45.5	
12-31	26.67	34.3	7.6	37.2	2.6	44.7	

the measurement of the total evaporation from the body in a given time has been used as a measure of the total heat loss (Root and Benedict, '26). It would seem that there must be considerably less error in using the excess temperature, and thus the heat loss by radiation and convection, as an index of the total metabolism, since we deal then with the 75 per cent of the total rather than with the 25 per cent lost by evaporation. The measurement of the average skin temperature over large areas of the body, as has been done by the author using a new technic described elsewhere (Burton, '34), gives a means of estimating very fairly the total heat loss from the body. As an example, table 1 is given, showing

how the excess temperature of the trunk of a subject lying on a bed in still air varied in the course of a fever² induced by administrations of 'Nirvinol.' At the height of the fever there was a 50 per cent increase in the excess temperature and therefore in the heat loss by radiation and convection. There was no significant change in the loss by evaporation in this period, sweating not occurring until after the fever had subsided. The metabolism was simultaneously determined by the Tissot-Haldane method.

The heat loss by evaporation is normally about 25 per cent of the total loss. Of this evaporation loss, about 36 per cent is lost from the lungs and the remaining 64 per cent from the skin. The normal loss is thus 25×0.64 , or 16 per cent of the total heat loss from the skin, while radiation and convection account for the remaining 75 per cent. We may then assume that the heat lost from an area of skin by evaporation is normally $16/75 = 0.21$ of the heat lost from that area by radiation and convection. Equation B may then be written as:

$$H = C (T_s - T_e) (1 + 0.21) = 1.21 C (T_s - T_e) \quad (C)$$

If we measure the evaporation H_e from the skin at the same time as we measure the skin temperature, and write H_{e0} for the normal value of H_e , which would give just 21 per cent of the total loss, we have—

$$H = C (T_s - T_e) (1 + 0.21 H_e/H_{e0}) \quad (D)$$

The application of this modification of the equation will be seen later.

The internal gradient. It was pointed out that the flow of heat from the interior of the body to the surface necessitated the existence of the internal 'physiological' gradient of temperature. This flow of heat takes place by the process of pure conduction across the tissues and by the transport of heat by the blood flow from hotter to cooler parts.

² These data were compiled with the cooperation of Mr. Rockwell at the Strong Memorial Hospital, Rochester, New York.

The laws of conduction of heat through a plane slab of material of infinitesimal thickness, whose faces are at different temperatures, are very simple. They are that the rate of flow of heat across the slab is proportional, a) to the cross-sectional area, A ; b) to the difference of temperature of the two faces ΔT ; c) inversely to the thickness of the slab, ΔX ; or in an equation—

$$H = K \cdot A \cdot \frac{\Delta T}{\Delta X}$$

$\frac{\Delta T}{\Delta X}$ is the thermal gradient, G , in degrees centigrade per centimeter. Here K is a constant for the material, the 'specific thermal conductivity.' Dividing by the area A , we have for the heat flow per unit area per unit time—

$$H = K \cdot G \quad (E)$$

The application of these laws to a case where there are finite thicknesses of material, not necessarily in plane slabs, and finite differences of temperature, involves the use of calculus and integration. The process is one very familiar to students of theory of heat, and is illustrated here simply to show its applicability to the flow of heat in living tissues.

Consider two proximate surfaces between which the heat is flowing, at the first of which the temperature is T_1 , the area A_1 and at which the gradient of temperature is G_1 . At the second surface the temperature is T_2 , the area A_2 (not equal to A_1 unless the heat is flowing in parallel lines across 'plane slabs') and the gradient is G_2 . Then the heat flowing in unit time into the section of material across the first face is—

$$H_1 = K A_1 G_1$$

or in the language of the calculus—

$$H_1 = -K \cdot A \cdot \frac{dT}{dX}$$

The heat that flows out of the section is—

$$H_2 = K A_2 G_2, \text{ or } H_2 = -(K A \frac{dT}{dX} + \frac{\delta}{\delta X} [K A \frac{dT}{dX}] dX)$$

These two must be equal unless there is retention of heat within the layer, which would therefore rise in temperature. In dealing with the 'steady state' only we exclude this term. If, however, heat is being generated within the layer, or amount, say 'h' Calories per second per cubic centimeter, the heat leaving the layer will be in excess of that entering it by the amount of the heat generated in the layer. The equation results:

$$H_2 - H_1 = -\frac{\delta}{\delta X} [K A \frac{dT}{dX}] dX = A. dX. h.$$

or

$$\frac{\delta}{\delta X} [K. A. \frac{dT}{dX}] = -A. h. \quad (F)$$

The integration of the equation when applied to bodies of various shapes gives the distribution of temperature throughout the body, i.e., the form of the gradients in the different parts.

The same mathematical analysis is adequate to describe the actual flow of heat in living tissues, where a major part of the flow may be through transport of blood. Such transport across the boundaries of the section may be resolved into two components, a flow in the direction of the heat flow at right angles to the faces, and one perpendicular to this. The former may be taken account of by considering that it effectively increases 'K,' the conductivity, that appears in the equation. The lateral flow which leaves heat in the section is mathematically equivalent to an increase in the heat generated in the section, the 'h' of the equation (F). The general results of the integration of (F) in special cases have therefore some application to the temperature distribution in the body.

The details of the integration in the case of bodies that have simple geometrical shapes, such as the cylinder and sphere, are to be found in almost any advanced textbook on heat. No useful purpose would be served by their repetition here. Some of the general principles that emerge may be cited as of interest in their application to the thermal gradients in the living body.

1. In a uniform medium in which heat flows in parallel lines, as through a slab where the temperature is uniform over a cross-sectional area, the drop of temperature is linear if no heat is generated in the medium itself. The gradient is therefore constant. For the same amount of heat flow per unit area of cross section, this gradient is greater the poorer the conductivity of the medium. The gradient thus tends to be

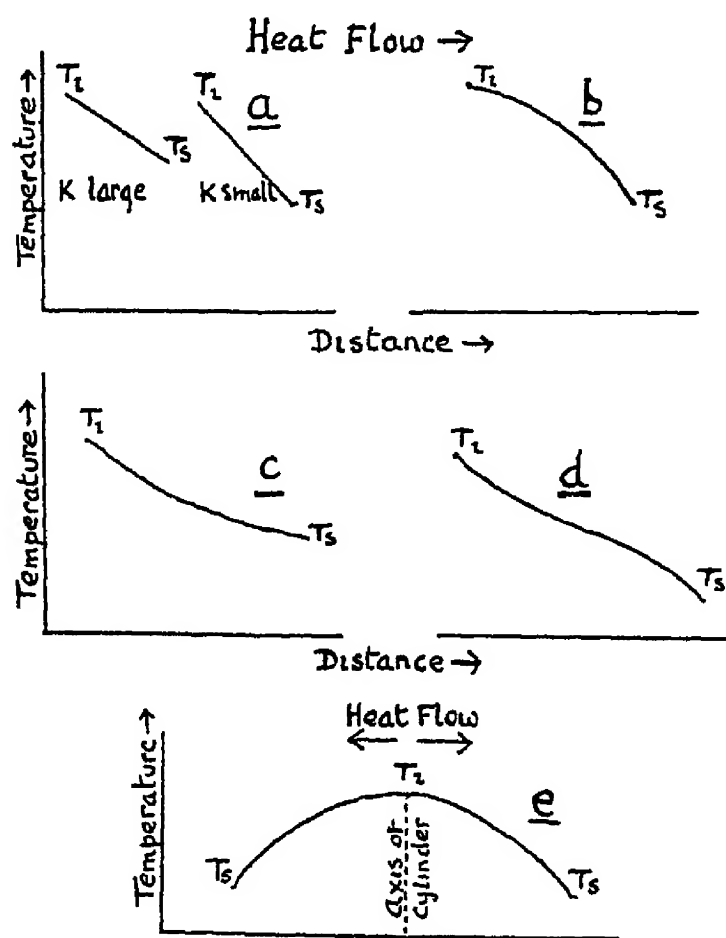


Figure 2

steeper in regions of small vascularity or of good thermal insulation, such as layers of adipose tissue. Figure 2 a illustrates this simple case.

2. If in addition to this 'passive' transfer of heat across the slab there is heat generated uniformly within it, the curve of temperature with distance becomes parabolic instead of linear, the gradient becoming steeper as we progress in the direction of heat flow (fig. 2 b).

3. When the heat flows, not in parallel, but in diverging lines, as when it is flowing from the center to the surface of a cylinder or sphere, the gradient is not uniform even if we have no heat generated. Due to the increasing cross-sectional area through which the heat flows the gradient becomes less as we pass outward to the surface. The case of a cylinder introduces a logarithmic term, that of a sphere, a term involving the reciprocal of the radial distance, but in each case the curve is of the form shown in figure 2 c.

4. When to the last case we add that heat is uniformly generated within the medium, a parabolic term is added as it was in b and the result is a complex curve as in figure 2 d. At the greater radial distances this latter term, the parabolic one, will predominate and the curve will tend to become convex upward, i.e., toward the direction of higher temperatures. In certain cases, as of a cylinder or sphere in which heat is generated uniformly throughout its volume, the equations become simpler, reducing a purely parabolic distribution of temperature (fig. 2 e).

We learn then that even in the case of a body of uniform thermal properties the distribution of temperature is not in general linear. The flow of blood and the generation of heat within the tissues tend to produce the parabolic type of temperature distribution, while the diverging nature of the flow tends to give the opposite curvature, particularly evident in regions of thermal inactivity. Temperatures at different depths in the human forearm and thigh have been measured by Bazett and McGlone ('27) and at different depths in the rectum and vagina by Benedict and Slack ('11). Their curves show that, as predicted, the distribution of temperature is in general parabolic. Had the analysis not been made, it might have been erroneously concluded that the increase of gradient near the periphery necessarily indicated a decreased conductivity in these regions. This may be the cause, but even in a perfectly uniform mass of tissue, the changing gradient would be evident if heat were generated in the tissues through which the heat flowed.

The equations give the value of the temperature T at a distance X , in terms of the quantities K , h , defined above, the temperature T_1 at some designated internal point, and the heat flow H per unit area across some designated surface. If for the distance ' X ' the linear dimension of the body, as ' R_s ' the radius of the cylinder or sphere, be substituted, the temperature T_s of the surface of the body results. ' H ' is then the heat flow per unit area at the surface. The results vary widely, of course, according to the details of the problem dealt with, but upon examination a characteristic common to all of them appears. It is that they can all be given the form—

$$T_1 - T_s = \frac{H R_s}{K} \cdot f \quad (G)$$

In this equation, f is a function whose form and magnitude depend upon the particular geometry of the problem chosen—the shape of the body, the position of the point where the temperature T_1 is stated, and so on. The importance of this result is that we may write—

$$H = C' (T_1 - T_s) \quad (H)$$

where C' is a coefficient of heat transfer (as was C in equation (A)) for the transfer of heat from the interior of the body to a point on the surface. In addition, we know that in bodies which are similar in geometry and specific thermal properties and which differ only in the size factor, this coefficient C' varies inversely as the linear dimensions.

$$C' = \frac{A}{R_s} \quad (J)$$

Though this has been deduced from consideration of a number of special cases, it can be shown to be a general theorem applying whatever the details of the problem chosen may be. Equation (J) will be used later in this paper.

An estimate of the order of magnitude of the thermal conductivity of tissue may easily be made. The measurements on the gradient of temperature in human subjects shows that there is a rise of temperature of about 5°C . (from 32° to 37°C .) to a depth of some 2.5 cm. The average gradient is

then about 2°C. per centimeter. For H, the heat reaching from the surface per square centimeter we may assume that the heat loss is the same as the average for the whole body, namely, about 40 kg.-Calories per square meter. Using equation (E) and reducing the quantities to the appropriate c.g.s. units, we find—

$$K = 0.0004 \text{ Cals./sq.cm./sec /}^{\circ}\text{C./cm.}$$

Comparing this with the values of the coefficient of conductivity K for common substances, we find that K for glass is 0.002, for water 0.0015, for wood 0.0004 and for flannel 0.0002. The tissues of the human body, in spite of circulation of the blood, are surprisingly effective as heat insulation. Lefèvre obtained values by direct measurement of the same order of magnitude for this quantity.

'*The thermal circulation index.*' We have equation (A), in which form the modified equation (B) can be written also:

$$H = C (T_s - T_e)$$

and equation (H)—

$$H = C' (T_i - T_s)$$

The heat coming up to a particular area of skin must equal the heat leaving that area to the surroundings. The two quantities H in these equations are therefore identical, and we have two simultaneous equations, from which we may eliminate one of the variables. The simple process of division eliminates H and we have—

$$\frac{C'}{C} = \frac{T_s - T_e}{T_i - T_s} = r \quad (\text{K})$$

The thermal conductivity of the underlying tissues may therefore be compared with that from the skin to the surrounding air by the calculation of this ratio. Under controlled conditions of humidity and ventilation the coefficient C may be made constant. Measurement of the skin temperature under these controlled conditions therefore enables us to follow changes in the coefficient C' which indicates the ease with which heat is transferred from the interior of the body to the area of skin chosen.

It has been recognized that the temperature of the skin depends on a great variety of factors, namely, the environmental conditions, the internal temperature of the body, the clothing, as well as upon the physiological conditions of the circulation. It is impossible to keep constant all these factors save the particular one that it is desired to study and thus the great quantity of data on skin temperatures to be found in the literature is hard to interpret. G. N. Stewart in one of his last papers ('30) remarked that there is no great difficulty in measuring the temperature of the skin, but that it was difficult to know what to do with the data once obtained. By the use of the ratio above, however, we eliminate at once the primary 'per se' influence of several factors, namely, of the environmental temperature, of the internal temperature and of the level of the metabolism. If the remaining factors that govern the coefficient 'C,' namely, the humidity, the ventilation and the clothing be controlled, we have left in this ratio a quantity that is an index of the physiological state of the tissues. Changes in the index will be chiefly due to changes in the peripheral circulation, while water and fat content will also play a part in determining its normal value. It may therefore be called a 'thermal circulation index.' An increase in its value indicates an increased conductivity for heat from interior to the surface of the body, which would be brought about by an increased peripheral circulation.

$$\text{Thermal circulation index, } r = \frac{\text{External drop of temperature}}{\text{Internal drop of temperature}}$$

The secondary influence of the other factors of environment and so on are of course not eliminated by its use, but their influence upon the physiological state of the tissues is shown by the change they induce in the index 'r.' In a warm atmosphere, for example, we should expect to find that the circulation index was increased. For a purely physical system, in which there could be no change in the coefficient C', though the surface temperature would of course rise in a warmer environment, the rise would be such that the value of the index would prove to be unchanged.

The numerical value of the index will be greatly different when calculated for different parts of the body, since it indicates the relative ease with which heat reaches the particular area of surface chosen, from the interior of the body where the hottest temperatures prevail. Its value depends upon the length of the path traversed by heat to that part, upon blood supply of the part, and upon the amount of superficial fat at the point. Table 2 gives temperatures taken by thermocouple measurements upon a subject after lying nude in an environ-

TABLE 2

Skin temperatures on subject (G McC) after lying nude for 20 minutes in room at 22.8°C. Rectal temperature = 37.25°C.

POSITION	SKIN TEMPERATURE °C	EXCESS TEMPERATURE °C	INTERNAL DROP °C	CIRCULATION INDEX
Forehead	33.40	10.60	3.85	2.75
Clavicle	33.60	10.80	3.65	2.96
Over breast	32.75	9.95	4.50	2.21
1 inch over umbilicus	34.20	11.40	3.05	3.75
Over apex of heart	33.30	10.50	3.95	2.67
Lumbar region	33.30	10.50	3.95	2.67
Arm, biceps	32.85	10.05	4.40	2.28
Palm of hand	32.85	10.05	4.40	2.28
Knee cap	32.35	9.55	4.90	1.95
Calf of leg	32.20	9.40	5.05	1.86
Sole of foot	30.20	7.40	7.05	1.05
Big toe	30.95	8.15	6.30	1.29

ment of constant temperature. In the calculation of the circulation index the rectal temperature is used as the 'internal temperature' for lack of a better. The values of the index indicate, for instance, that heat from the interior reaches the surface of the abdomen three times more easily than it does the sole of the foot.

In order to make studies of circulatory changes a particular area of skin, such as that of the trunk is chosen, and the changes in the index for that area observed. If changes in the evaporation are suspected, the index may be corrected for these if the evaporation from the area is measured and com-

pared with the normal evaporation. For, using the modified equation (D), we would obtain—

$$r = \frac{C'}{C} = \frac{T_s - T_e}{T_i - T_s} \left(1 + 0.21 \frac{H_e}{H_{eo}} \right)$$

The uncorrected values of the index are then to be modified by multiplication by the factor $(1 + 0.21 \frac{H_e}{H_{eo}})$. An increase in the evaporation to double the normal amount by sweating would thus produce an error in the apparent circulation index of about 20 per cent only. In the preliminary work reported here, these corrections have not been made.

Variability of the index. Preliminary studies of the skin temperatures over the trunk and over the leg of a normal subject indicate that the normal variability of the circulation index, in quite a wide range of environmental temperatures, is of the order of 20 per cent for the trunk, and 30 per cent for the leg.

In abnormal conditions of the circulation, however, as in fever, there are very much greater changes than this. The seventh column of table 1, for instance, indicates how in the height of the fever and erythema of the skin the index had increased some four- or fivefold. In a case of severe edema (hives) without fever, the index showed a two- or threefold increase, presumably due to the increased thermal conductivity of tissues containing water.

It is well known that under local or general anaesthesia there is practically complete vasodilatation of the peripheral vessels which is accompanied by a rise of temperature of the skin, particularly of the extremities. Morton and Scott ('30), among others, have used this as an indication of sympathetic vasoconstrictor activity. Some of their results are given in table 3.

TABLE 3

	ROOM TEMPERATURE °C	TOE TEMPERATURE °C	CIRCULATION INDEX	FACTOR OF CHANGE
Initial	25.4	27.0	0.16	
After NO ₂ + O ₂	25.4	28.5	0.36	2.2
After ether	25.4	33.0	1.90	12.0

Rectal temperature is assumed to be 37.0°C.

Under complete anaesthesia the index for the toe changed by a factor of twelve times. This large change is not as surprising as at first sight when Poiseuille's law of the flow of liquids through a tube of small diameter is referred to. It states that increase of diameter of the capillaries to twice their former size would result in a sixteenfold increase in the blood flow through them and through the superficial skin. (Flow varies as fourth power of diameter.) Changes of diameter of capillaries of much greater magnitude than this are known to take place. The work of Stewart, of Pickering, of Grant, Bland and Camp ('32) and others, on the vascular reactions of the rabbit's ear, is of interest in this connection. Their results show variations in the circulation index of similar magnitude. Incidentally the usefulness of the index is here illustrated. The ear is first immersed in a water bath at say 15°C. and its surface temperature noted by the use of thermocouples. The temperature of the water bath is then changed to say 27°C. and the temperature of the surface of the ear is found to rise. Whether this rise is simply that which would naturally follow in a purely physical system, or whether it involves a vascular response may be discovered by calculation of the index in the two cases. An increase in the index means that there has been vasodilatation; a decrease, vasoconstriction.

The dependence of skin temperature upon environment and body temperatures. Equation (K) may be rearranged to express the dependence of skin temperature upon the environmental temperature T_e and upon the internal temperature T_i . It yields

$$T_s = \left(\frac{1}{1+r} \right) T_e + \frac{r}{1+r} T_i \quad (L)$$

Talbot ('31), in his studies on the skin temperatures of children, remarked upon the fact that the temperature of the extremities was very sensitive to changes in environmental temperature but relatively insensitive to changes in body temperature, while of the temperature of the chest the reverse was true. That this follows from the equation above is shown

by table 4, which is constructed directly from that equation assuming convenient values for room and for internal temperatures. The extremities are at temperatures of about 28°C. while the chest may be taken to be at about 33°C. in such circumstances.

Vincent (1890) from his studies derived a correction factor which should be subtracted from the skin temperature to reduce all values to a standard room temperature, which was the 20°C. used in the above table. The correction he gives as 0.3°C. per degree rise of room temperature, the equation being—

$$T_s = 26.5 + 0.3 T_r.$$

This is the value predicted by the table for skin temperatures at about 31°C.—a good average value for the skin temperature over the body. Reichenbach and Hegmann ('07) give a formula for the dependence of the skin temperature (H) upon air temperature (L) and upon internal temperature (B).

$$H = \frac{B}{K} + \frac{K-1}{K} L$$

where K is a numerical constant. In the symbols used in our equation this is—

$$T_s = \frac{K-1}{K} \cdot T_e + \frac{1}{K} T_r$$

The equation is identical with that derived from pure physical principles if K is put equal to $(1 + \frac{1}{r})$. These authors give the physical derivation in much the same way as it has been developed here, but do not note that the dependence of skin temperature upon room temperature itself depends upon, and can be predicted from (as in table 4) the level of the skin temperature at a particular room temperature.

TABLE 4
Room 20°C., rectal 37°C.

SKIN TEMPERATURE	INDEX C°/C	CHANGE FOR 1° ROOM	CHANGE FOR 1° RECTAL
°		°C.	°C
35	7.5	0.18	0.88
33	3.3	0.23	0.77
30	1.4	0.42	0.58
28	0.90	0.47	0.53
25	0.42	0.70	0.30
23	0.21	0.83	0.17

It is well known also, that there are greater fluctuations due to circulatory changes, in the temperatures of the extremities than of the warmer parts of the skin. This also may be deduced from the equation (L) by tracing the dependence of skin temperature T_s upon the index r , the other factors remaining constant. Table 5 gives the results. A

TABLE 5
Per cent change in index for 1°C. in skin temperature =

$$\left(\frac{1}{T_s - T_c} + \frac{1}{T_i - T_s} \right) \times 100$$

SKIN TEMPERATURE °	INDEX C°/C	PER CENT AGE CHANGE IN INDEX FOR 1° CHANGE IN SKIN TEMPERATURE	
35	7.5	57	
33	3.25	33	Room 20°C.
30	1.43	24	Rectal 37°C.
28.5	1.00	12	
27	0.70	22	
25	0.42	28	
23	0.21	40	

change of 1°C. in skin temperature of the chest indicates a far greater change in circulation than an equal change of temperature of the extremities. The maximum sensitivity of skin temperature to circulatory changes occurs when the skin temperature is just halfway between the environmental and internal temperatures. The temperature of the extremities happens to lie nearer this maximum condition than does that of the chest. It is not justifiable, therefore, to conclude from

the greater variation in temperature of the extremities that circulation changes are greater here, although this may be true. Reliable simultaneous measurements made upon skin temperatures of different parts of the body under different environmental temperatures would be necessary to decide whether vasometer adjustments, as in the heat regulation of the body, are general or localized in particular parts of the body. The results of preliminary experiments upon the skin temperature of the chest and of the leg in different environmental temperatures have so far indicated that the index changes very little, if at all appreciably, in the range of temperature from 20 to 30°C. If this is confirmed it means that the part played by dilatation of the peripheral vessels in the

TABLE 6

J. Stewart, weight, 29.2 kg. Height, 131 cm. Age, 8 years

DATE	ROOM TEM- PERATURE	SKIN TEM- PERATURE	EXCESS TEM- PERATURE	RECTAL TEM- PERATURE	CIRC- ULATION INDEX	METABOLISM CAL/S /SQ M	REMARKS
3-5-33	22.28	33.40	11.20	37.5	2.7	43.8	No skin reaction
3-6-33	23.15	32.15	9.0	37.0	1.85	45.1	
3-7-33	27.46	33.96	6.50	37.4	1.9	45.6	
3-8-33	24.52	33.11	8.59	37.2	2.1	48.4	
3-9-33	19.38	31.25	11.87	37.5	1.9	42.4	

regulation of heat loss in man at any rate in the normal range of environmental temperatures, has been greatly overestimated. As an example, table 6 is given. In this case the subject did not react to the drug (Nirvinol) and no fever or skin reaction was evident. The constancy of the index in widely different room temperatures is remarkable when compared to the variation shown in table 1, where fever was induced. Lefèvre (loc. cit., p. 399) found that the conductivity of the superficial layers of the skin was twice as great when the subject was immersed in a bath at 30°C. as when in one at 5°C.—these, however, are extreme limits of temperature. The normal level of the average skin temperature being about 32°C. in surroundings of say 24°C., the excess temperature is of the order of 8°C. Even if the conductivity

were increased to an infinite value the skin temperature could not rise to a value above the body temperature, 37°C. The excess temperature would then be increased to 13°C.—an increase of only some 50 per cent. Vasodilatation is, therefore, quite inadequate to deal with the increases of 200 to 1000 per cent in heat loss that are necessary in exercise. Here sweating must be, and is, called upon to get rid of the excess heat by evaporation.

Metabolism and environmental temperature. Returning to the two fundamental simultaneous equations (A) and (H),

$$\begin{aligned} H &= C (T_s - T_e) \\ H &= C' (T_i - T_s) \end{aligned}$$

we may eliminate the skin temperature T_s instead of the metabolic heat loss per unit area, H . The result of this elimination is the equation:

$$H = C \frac{(T_i - T_e)}{1 + \frac{C'}{C}} = C \frac{(T_i - T_e)}{1 + \frac{1}{r}} \quad (M)$$

In this equation 'C' and 'r' refer to the external coefficient of heat transfer and the circulation index, respectively, for the particular area of surface chosen, while H is the heat loss, say Calories per square meter per hour, from this area. A relation of the same form as (M) will hold between similar quantities which are average values for the whole surface of the body. H will then be the average heat loss in Calories per square meter per hour, the quantity (equal in the long period of time to the heat production) which is measured in studies of metabolism.

Equation (M) emphasizes the fact that if the metabolism of the body changes there must be a compensating change in the quantities on the right hand side of the equation. Unless the environment and clothing are changed, an increase in the metabolism must result in a change in the internal temperature T_i , if there be no compensating change in the index 'r.' Changes in circulation must accompany changes in the level of metabolism if the body temperature is to re-

main within the limits suitable to life. The increase in the index found in fever is an indication of this compensating change of circulation. Similarly, when the environment is changed, ' T_e ' changing, either the metabolism must change so that the internal temperature may remain constant, the 'chemical regulation' of Rubner, or the index r must change by a change of circulation, sweating or peripheral dilatation or constriction. The latter is the 'physical regulation.' The equation therefore expresses in a concise form these considerations.

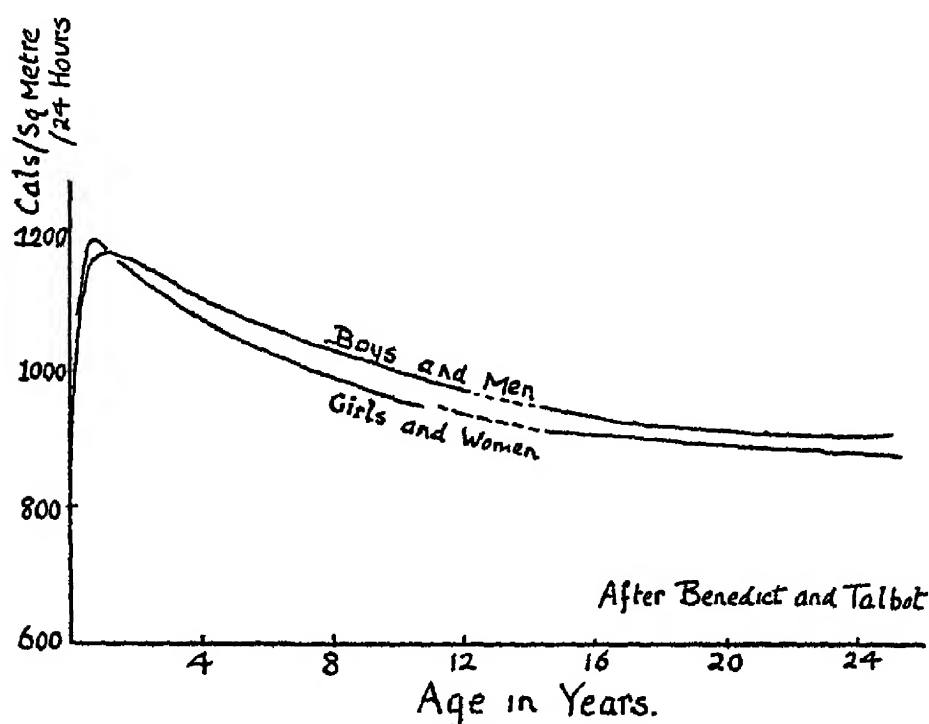


Figure 3

Metabolism and size. When equation M is applied to the consideration of the changes in metabolism per unit area during growth, interesting conclusions are indicated.

In the well-known human 'age curve' (fig. 3) the metabolism per unit area is plotted for different ages. The accepted curve is the result of averages of a great number of determinations of basal metabolism upon a number of subjects by Benedict, Murlin, DuBois, Boothby and Sandiford and others. For the average curve that results, we may justifiably assume that the temperature of the environment, T_e , was the

same for all ages, although this factor has not been controlled in experiments upon normal basal metabolism as well as would be desirable and there must have been considerable variation in the individual cases from which the average curve was derived. The coefficient C we may assume also to be sensibly constant. If the environment were colder, making the difference $(T_1 - T_e)$ greater, the tendency would be to increase the clothing of the subject for him to be 'comfortable' and thus decrease C for this particular case. The tendency is then for variations in the two factors T_e and C to compensate so that on the average the result is constancy. There remains the possibility that children are habitually clothed more warmly, or perhaps, less warmly, than adults so that C is not constant at different ages. This cannot be denied, but it does not seem likely in view of the fact that the scatter of individual points is not greater than it is. The regulation mechanism, by which the metabolism is rendered relatively constant in spite of changes in the amount of clothing also tends to eliminate the changes of this factor. In the case of infants the habitually greater clothing may play some part. What follows is based on the assumption that the same type of curve would have been obtained if the metabolism had been measured on all subjects lying unclothed in rooms of a standard temperature. The remaining factor of the numerator of (M) , the internal temperature, is on the average the same—about 37°C . for all ages. For young children it is slightly higher, 37.5°C ., but the difference is insignificant in view of the large changes in the metabolism curve. These changes may then be referred to a change in the normal value of the thermal circulation index ' r ' that occurs in the denominator of the fraction in (M) .

As the body increases in size, the absolute amount of tissue through which the heat must be transferred to reach the surface increases. It was shown that, if the thermal nature of the tissues and circulation remain the same, for bodies that are thermally and geometrically 'similar' this size factor changes the value of the coefficient C' by the equation (J)

$C' = \frac{A}{R_s}$. Thus the circulation index, $\frac{C'}{C}$ for such 'similar' bodies would vary inversely as the linear dimensions of the body. Thus we may rewrite equation (M) as—

$$H = \frac{B}{1 + aL} \quad (N)$$

'L' being a linear dimension of the body. 'B' and 'a' are constants. The metabolism per unit area of the smaller body would (for such 'similar' bodies) be therefore greater. In simple terms, the smaller body has less insulation in absolute amount. There is then less drop of temperature from interior to surface, and, the internal temperature being the same, the average skin temperature is higher, so that the heat loss and the metabolism per unit area are therefore greater. The experimental observation is that after the maximum at the age of $1\frac{1}{2}$ to 2 years, the metabolism per square meter does actually decrease with increasing size.

For an estimate of how great a decrease the size factor would produce on the heat loss per unit area, we may take the cube root of the weight as proportional to the linear dimensions of the body, writing the equation—

$$H = \frac{B}{1 + A'W^{\frac{1}{3}}} \quad (O)$$

The value of the constant A' is known if we know the value of the average circulation index at any given weight. If, for instance, we assume a thermal circulation index of 2 for 70 kg. man, which means that his average skin temperature is about 31°C . in an environment at 20°C ., we have, since $A'W^{\frac{1}{3}} = \frac{1}{2}$

$$A' = \frac{1}{2 \times \sqrt[3]{70}} = 0.121$$

and

$$H = \frac{B}{1 + 0.121 W^{\frac{1}{3}}}$$

Substituting the measured value for H, 925 Cals./sq.meter/24 hours, for the weight 70 kg., we obtain the value of B.

$$B = 1388, \text{ and } H = \frac{1388}{1 + 0.121 W^{\frac{1}{3}}}$$

Thus it is possible, knowing the level of the metabolism at a given weight and the average circulation index, which involves a knowledge of the average skin temperature over the body in surroundings of a given temperature, to predict what the metabolism per unit area would be in bodies that were 'similar' but of other weights.

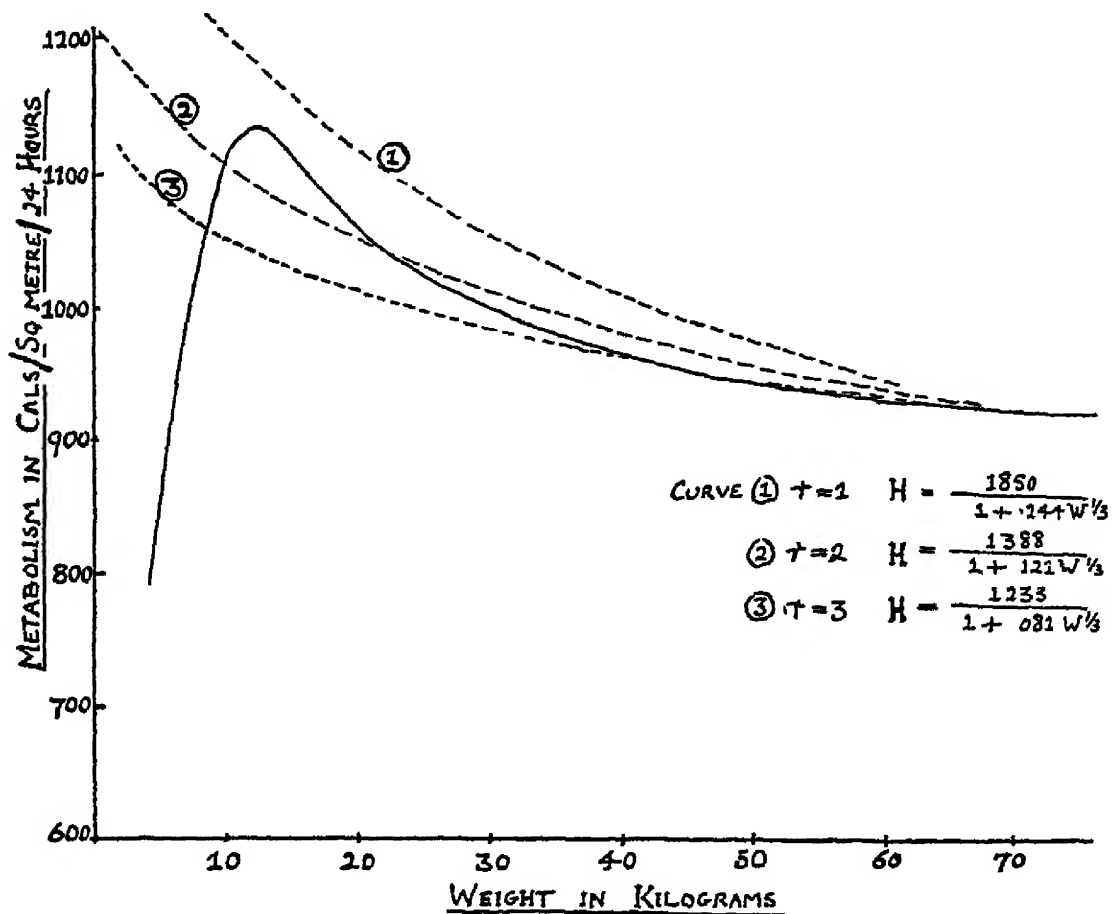


Figure 4

In figure 4 is plotted in the continuous line the experimentally determined curve of metabolism per square meter against weight, after Benedict and Talbot ('21). On the same chart the dotted lines represent the result of the size factor, calculated as indicated above, assuming the different values 1, 2 and 3 for the circulation index 'r' of the 70 kg. man. It is seen that for an index between 2 and 3, the fall of the metabolism per unit area, after the maximum that occurs at about 15 kg., is of about the same order as would be produced

by the size factor alone. The scatter of the points from which the experimental curve was deduced are so great near the maximum that the true curve might well coincide with the calculated curves from that point on. It is therefore possible to attribute the progressive decline in the metabolism per unit area after the maximum that occurs at the age of 1 or 2 years to the increase in the absolute amount of thermal insulation of the body with increasing size, the thermal nature of that insulation (its specific conductivity K) remaining constant. Confirmation of this view of the role of thermal insulation is found in the fact that in the age curve for men the metabolism per unit area becomes constant, or attains a much smaller slope, at just the age when growth ceases. In women, however, the level is consistently lower and continues to fall until later life. This is consistent with the known greater thermal protection of fat in women and its continued increase until middle life.

Below this critical weight that gives the maximum, however, there is a wide departure between the observed and predicted curves. In spite of the fact that its size is increasing, and with it, if it were a 'similar' body, the absolute amount of its thermal insulation, the metabolism of the infant per unit area increases rapidly from birth. The conclusion is inescapable that for the infant either the amount of insulation relative to its linear dimensions is much greater or the insulation is of a much more effective kind. An estimate of how great a change in the thermal properties of the insulating tissues would be necessary, may be made on the assumption that the relative amount is the same, varying as the cube root of the weight, as was assumed for the rest of the curve. The whole change is then attributed to the change in the coefficient of conductivity ' K ' which occurs, by equation (G), in the coefficient C' as $\frac{B}{K}$. Equation (O) is then written—

$$H = \frac{B}{1 + \frac{bW^{\frac{1}{3}}}{K}}$$

Where b is a constant. If we know that the circulation index is r at the adult weight (70 kg.) at which the metabolism is H_0 we have also

$$H_0 = \frac{B}{1 + \frac{1}{r}}$$

Solving we obtain

$$K \propto \frac{W^{\frac{1}{3}}}{\left(1 + \frac{1}{r}\right) \frac{H_0}{H} - 1}$$

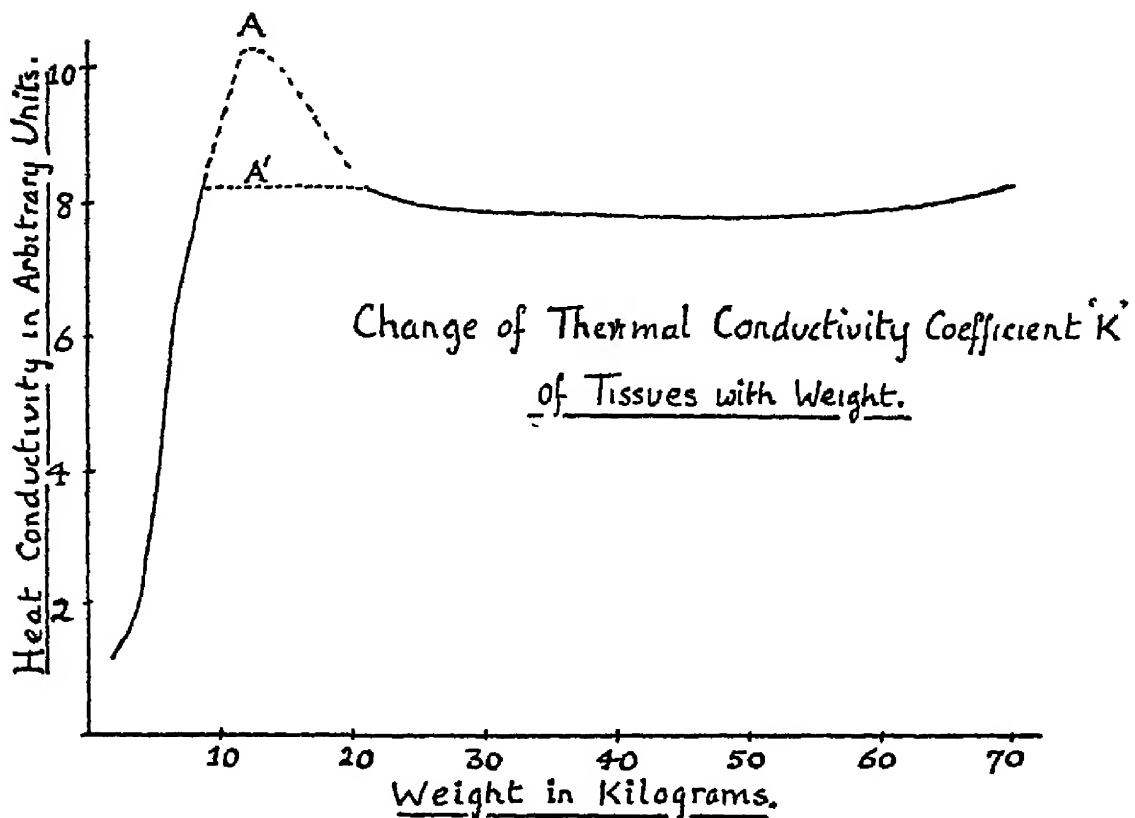


Figure 5

The changes in 'K' thus deduced from the experimental curve of metabolism are shown in figure 5, where the index r for the adult is chosen as $r = 2$. It is seen that the insulation of the infant must be some five or six times as effective as that of the adult, and that there is a progressive change in conductivity until the age of $1\frac{1}{2}$ to 2 years, after which the conductivity is sensibly constant. The peak at A cannot be taken to be significant, since it is the peculiarity of the equation that in this region a change of only 4 per cent in the

metabolism per unit area, H , would bring the curve for K down to A' . It is not claimed that the experimental curve of figure 4 has a certainty of anything like this 4 per cent, especially in the region referred to. In the region of lower weights, however, a much greater change in the metabolism curve would be necessary to change the curve of figure 5 very much. Very little change in the general conclusions is made if instead of $r=2$, $r=1$ or $r=3$ is taken. It is difficult to assign a good average value for the skin temperature over the whole body under the 'normal' clothing in 'normal' conditions, but in a room temperature of 20°C . that average temperature, from the data in the literature, lies probably between the limits of 27.5°C . and 33°C ., which would correspond to values of the circulation index ' r ' of 1 and 3, respectively.

For this progressive increase in the thermal conductivity of the tissues in the early years of life, two explanations may be suggested. It may be that there is a decrease in fat and an increase in water content of the growing infant. Since water has a thermal conductivity that is three or four times the average thermal conductivity of tissue, and fat considerably less conductivity, this would produce a progressive increase in the conductivity. This influence of fat in controlling heat loss and thus heat production in atrophic and in normal infants has been discussed by Murlin ('23). A second contributing factor, which may be of major importance, would be a progressive increase in the peripheral and general circulation of the growing infant.

It must not be forgotten that, as has been mentioned, the fact that infants are habitually clothed more completely than in later life might vitiate these conclusions to some extent. It is difficult to believe, however, that clothing could change the external coefficient C , and therefore the index $\frac{C'}{C}$ by a factor as great as six times. An experiment upon the skin temperature on the chest of a subject, both nude and fully clothed indicated that the index was increased by about 80 per cent by the full clothing of vest, shirt and coat. It is not conceivable then that differences in the amount of clothing

at different ages would produce changes as great as the metabolism curve necessitates. The reality of these conclusions is substantiated when the trend of metabolism per unit area is examined in other species than the human.

Age curve of metabolism in other species. Brody and his co-workers ('30) have followed the basal and 'resting' metabolism of farm animals from birth to maturity. They found that for dairy and for beef cattle, for pigs (including the results of Deighton), and for chickens the curves are very similar to those for man. The metabolism per unit area, starting at a low level, rapidly rises to a maximum in early life after which it declines slowly to maturity. The maximum occurs at a period which corresponds to the weaning time of the animal (in the chick it is at about 30 to 40 days of age). In the case of the colt, however, there is a continuous decline of the metabolism per unit area from birth. The final decline with increasing size appears to be a general rule, which would be predicted (from the influence of the size factor) if there was physiological and thermal similarity. Table 7 gives the results for different species as far as they are available. The figures must be accepted with a great deal of caution and are cited only to represent the trend of metabolism with growth.

TABLE 7
Metabolism per square meter per day

SPECIES	LEVEL AT BIRTH	MAXIMUM	AGE FOR MAXIMUM DAYS	FINAL ADULT LEVEL
Cattle	1200	2000	200	1700
Swine	1300	2400	250	1200
Fowl	600	1400	35	920
Horses	2600	No maximum		950
Man	650	1150	1½ years	925

It will be noted that the final decrease to the adult level is considerably less in the case of cattle than in that of swine and of horses. This is what might be expected if this decline is attributable to the increasing amount of insulation, for the high final level of the metabolism per square meter of cattle

indicates that the insulation in their case must be less effective than that of other species. If this is so, increase in the amount of this insulation would not be expected to produce as much change as increase in the amount of more effective insulation such as that of the pig.

Brody ('30) after examination of the metabolism during growth of a number of different species, including men, cattle, sheep, horses, swine, dogs, guinea pigs, rats and some birds, concludes that for all the metabolism per unit weight may be well represented by an equation of the form—

$$Q/m = Ae^{-km} + C$$

where Q is the metabolism per unit time, ' m ' is the weight of the animal and A , K and C are constants. He also shows from actual measurements of surface area of animals that the surface area follows a law—

$$S = Bm^n$$

where B and n are constants for a particular species, varying of course from one species to another. (Meeh's formula for surface area assumes $n = 2/3$.) Combining the two equations to find the metabolism per unit area instead of per unit weight, we find—

$$H = Q/S = \frac{(Ae^{-km} + C) m^{1-n}}{B}$$

Examination of the variation of the quantity H with increasing weight, by the methods of the calculus upon this equation, shows that it will follow just such a curve as has been found to hold for metabolism per unit area, there being an initial rise to a maximum and a subsequent more gradual decline. The maximum is predicted by the calculus to occur at a weight given by—

$$m = \frac{1}{k} \left[(1-n) + \frac{C}{A} (1-n) e^{1-n} \right]$$

approximately.

It is quite indefensible to use the results of calculations made from empirically fitted mathematical laws unless it is

evident that no extrapolation is involved to regions beyond the experimental range. Thus it cannot be concluded that since the form of the equation for the metabolism per kilogram is a general one for all species, the maximum exists for all species. For instance, for man the constants of the equations given by Brody are $k=0.035$, $n=0.7$, $c=20$, $A=50$. These give the weight at which the maximum occurs as 13 kg. Actually it is found to be at about 10 kg. Whereas, from the equations given for the horse, $k=0.008$, $n=0.63$, $c=22$, $A=60$ we find the maximum predicted at a weight of 90 kg., which is far below the range of the experimental values to which the equations are fitted and has therefore no significance. Inasmuch, however, as the form of the equations seems to be generally applicable for the later ages, the eventual decline of metabolism per unit area with increasing size appears to be a general law.

Biological implications; the surface area law. Since Rubner pointed out the constancy among animals of very widely different size and species of the metabolism per unit area, a great deal of discussion and controversy has ensued whether this 'surface area law' has a causal significance, the heat loss determining heat production, or whether it is not to be regarded as a mere coincidence, some other factor as the 'mass of active protoplasmic tissue' of Benedict ('15), which happens to be proportional to the surface area of the animal, is the determining factor. Even the most decided opponents of the causal significance, however, cannot deny that on the average heat loss must be balanced by heat production, if the body temperature is to be maintained.

It may well be argued that the emphasis should be shifted to the consideration of the last phrase; to the fact that of the varying characteristics of growth the body temperature is one that remains at almost unvarying level. The existence of the complicated and very effective mechanism of heat regulation in the homoiotherms for the maintenance of the body temperature within narrow limits suggests that its constancy is something fundamental and essential to the animal.

Van't Hoff's law may apply to isolated chemical processes, but it very evidently does not hold for the complex of linked chemical and nervous processes that forms the whole organism. Metabolism increases with the temperature up to a certain point but beyond a certain limit there is instead a decrease, which, if irreversible, results in death. If metabolism increases in fever, the regulating mechanism eventually increases the heat loss by sweating and vasomotor adjustments so that the temperature is restored to normal. This means that there is a change in the thermal properties of the tissues, of the coefficient of heat transfer from interior to surface, which in the case of fever is temporary, lasting only until the body temperature is restored. If we turn to consider increases in metabolism that result from growth of the animal, we realize that if the accompanying increase in surface area is not adequate, there must be compensating changes, permanent in this case, of the thermal properties of the insulating tissues. The necessity of the elimination of the heat produced in order that the optimum temperature for life may be maintained, links together the thermal properties of the body and its thermal activity, and thus its metabolism. The one is a function, in the mathematical sense, of the other.

Which of these two characteristics, the metabolism or the properties of heat transfer, is the controlling factor and which is the controlled, seems to be a question for philosophy rather than for physiology. The observed facts shown by the 'age curve' of metabolism indicate that at first the metabolism increases very rapidly, even more rapidly than the weight, and that the thermal properties of the body tissues change rapidly to compensate. In later life the specific thermal properties of the tissues remain the same, while the metabolism per unit area decreases in accordance with this fact. Of the three connected variables, the thermal activity of the body, the thermal properties of the body, and its temperature, the last seems from its constancy throughout to be the dominating one. Of the remaining interdependent factors, the second, that of the thermal properties, seems to reach

constancy eventually though not in the early stages of growth. Finally, when growth has ceased at maturity, all three factors remain relatively constant.

SUMMARY

The fundamental law of heat flow demands that there be an internal 'physiological' gradient of temperature from the interior of the body to an area of surface as well as an external physical gradient from that surface to the surroundings. The laws governing flow of heat down these gradients are considered. Newton's law applies to the loss of heat from the skin by convection, radiation and conduction, the loss by evaporation being considered separately. The application of the laws of conduction to the transport of heat through the underlying tissues to the skin is shown and the conclusion is reached that the distribution of temperature with depth will be in general, as it has been found, parabolic. A 'thermal circulation index' is defined by which from measurements of skin temperature, changes in the properties of heat transfer of the tissues may be studied. Examples of such changes are given. The dependence of the skin temperature upon internal temperature, upon environmental temperature, and upon the circulation is deduced.

On the assumption that there is thermal 'similarity,' i.e., that the factor of absolute size is the only variable in growth, a relation is deduced for the variation of metabolism per unit area with increasing weight. Comparison with the 'age curves' of metabolism in humans shows that the eventual decrease from an early maximum to the adult level is consistent with an increase in absolute amount of the thermal insulation, its relative amount remaining constant. From birth to the maximum at $1\frac{1}{2}$ years, however, there must be a progressive decrease in the relative amount or in the effectiveness of the insulation.

The trends of metabolism per unit area in other species are discussed and conclusions are drawn as to the interpretation of the surface area law in view of the fact that the body

temperature, the thermal properties of the tissues, and the thermal activity of the body are interdependent.

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POSSIBLE SOURCES OF CALCIUM AND PHOSPHORUS IN THE CHINESE DIET

I. THE DETERMINATION OF CALCIUM AND PHOSPHORUS IN A TYPICAL CHINESE DISH CONTAINING MEAT AND BONE

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That milk is used only for infants and invalids is not merely a prejudice but a dietary practice quite universal among the Chinese. The absence of milk and dairy products in the native Chinese diet has drawn continuous interest because of the sharp contrast in the diet of the occidental world where milk is used freely. This fact has led to an inquiry regarding the source of calcium particularly in the Chinese diet. Soy bean and some of its products have been found to be high in calcium and phosphorus. Definite information is lacking as regards the physiological utilization of soy bean as the main source of supply of these elements. Pittman ('32) has found that even an intake of 4 ounces, dry weight, of navy beans daily was unable to maintain a calcium balance. However, Adolph and Chen ('32) have reported that an equal calcium intake supplied by cows' milk or soy bean curd produced the same effect on calcium equilibrium for the Chinese adult. In addition to the liberal supply of vegetables usually provided the Chinese use fowl, pork and fish as commonly in the diet as Americans use milk and cheese. Recognizing the low content of calcium in meat in contrast to milk, the question arises as to the availability of calcium from the bone in such meat dishes as effected by the method of preparation and way of eating.

A commonly used, cheap dish, 'sweet-sour pork spareribs,' may serve as an illustration. Spareribs are cut up in 1½-inch pieces, slightly browned and cooked slowly until very tender with a large amount of diluted vinegar, soy bean sauce, and sugar to taste. The excess liquid is thickened with starch before serving. Due to the long period of cooking, the solution penetrates the soft bone marrow. It is a satisfaction for those who dine with the family to suck and chew the small pieces of bone while eating. A similar dish but with a large proportion of bones, usually pig's feet, ginger and a high concentration of vinegar is prepared for the lactating mother as a main dish in three or four meals a day, together with eggs, a few vegetables, and occasionally other meats as supplements. This diet is started right after childbirth and lasts 30 to 40 days. It is generally believed that this is good for milk production, blood regeneration and general vitality. Such a time-honored custom, while it appears to lack any scientific support, seems worth investigation to determine the actual amounts of calcium and phosphorus obtained from the consumption of such a combination of bone, meat and sauce.

This study is devoted only to the quantitative measure of the possible amounts of calcium and phosphorus obtained from this particular preparation of meat and bone. The forms in which these elements might be found were not determined nor were biological metabolic tests made to determine the absorption and utilization of such elements in the human or animal body.

EXPERIMENTAL

Standardization of the preparation of 'sweet-sour spare-ribs.' To provide a typical Chinese meat-bone dish for analysis, the recipe and method of cooking were tested until the finished product resembled the native home-made dish. The standardized formula for an individual serving is as follows:

Pork spareribs	150 to 180 gm.
Cooking solution (total)	250 cc.
Rice-vinegar	75 cc.
Soy bean sauce	10 cc.
Distilled water	150 cc.
Sugar	10 gm.
Salt	1 gm.
Thickening	
Cornstarch	5 gm.
Distilled water	15 cc.

Pork is the only kind of meat used in this dish. To make it more typical, imported Chinese rice-vinegar and soy bean sauce were used. This vinegar is an acid product resulting from the fermentation of rice. Soy bean sauce is well known as the chief seasoning in Chinese cookery. Since it is made from fermented soy beans the question has often been raised whether or not it furnishes additional calcium and phosphorus in the Chinese diet.

Sampling. In preparing samples for this experiment, the spareribs of one side of the animal were purchased from the market. The middle six ribs were chosen and each cut into six 1½-inch pieces. Alternate pieces of each rib were taken to make two rather uniform samples. One of these samples was cooked in a small enameled kettle, over an electric plate, starting with a medium temperature. The meat was browned slightly and then the cooking solution was added, covered and cooked at low temperature with little stirring until the meat was tender and came readily off the bone. This took from 50 to 60 minutes. The remaining liquid was thickened with the cornstarch paste. After it boiled again the heat was turned off and the contents allowed to stand for 15 minutes, the time considered used in waiting to be served. The total time for preparation was 80 minutes, 75 of which the meat and bone were in contact with the solution. The meat was separated from the bone with a knife and fork, then washed with hot distilled water and drained. All visible pieces of bone were collected and treated likewise. The solution together with the washings of the meat, bone and utensils was collected. For the raw sample, the bone was separated as

completely as possible from all the meat fibers. Below is a list of samples taken for analysis in the preparation of the prepared meat-bone dish.

<i>Bone series</i>	<i>Meat series</i>	<i>Solution series</i>
Raw bone	Raw meat	Soy bean sauce Rice-vinegar
Cooked bone	Cooked meat	Cooking solution Cooking solution (cooked)

Methods of calcium and phosphorus determinations. The calcium was determined by precipitation as calcium oxalate and titration with standard KMnO_4 solution. The precipitation was made in the presence of ammonium chloride, in a hot solution which had been made pink to methyl red by the addition of acetic acid. The calcium oxalate was titrated under conditions suggested by the U. S. Bureau of Standards ('20 and '12) in which the standardization of KMnO_4 solution was made with sodium oxalate.

Phosphorus was determined by Pemberton's alkalimetric method (1895). The phosphorus was precipitated as ammonium phosphomolybdate. The yellow precipitate was dissolved in an excess of standard NaOH solution. The excess NaOH was back titrated with standard HCl . This is similar to a method given by the Association of Official Agricultural Chemists ('30).

RESULTS

Calcium and phosphorus in the cooking solution. To check the possible sources of calcium and phosphorus the rice-vinegar, soy bean sauce and the combination of all the ingredients in the cooking solution used in the recipe were analyzed for these elements. The results are recorded in table 1. The total calcium of the cooking solution was only 2 mg. more than the sum of the calcium from vinegar and soy bean sauce. The total phosphorus was somewhat higher than the sum of the quantities in the two ingredients. Regardless of this fact, the actual amounts of calcium and phosphorus in the cooking solution were taken into consideration in the final results.

The titratable acidity of the cooking solution was determined, giving a normality range of 0.150 to 0.152. The pH value as measured by means of a hydrogen electrode was 3.20 to 3.24.

Solubility of tricalcium phosphate in water and cooking solution. Four samples of tricalcium phosphate were weighed out. Two of these samples were cooked in 167 cc. of the cooking solution, as described in the recipe, for 1 hour. The others were kept in the same amount of water as the cooking solution for the same length of time. A clear solution for the determination of solubility was obtained by centrifuging. The results are shown in table 2. The percentages of solubility

TABLE 1
Calcium and phosphorus in the cooking solution

INGREDIENTS	SAMPLE	CALCIUM	AVERAGE CALCIUM	PHOSPHORUS	AVERAGE PHOSPHORUS
	cc	gm	gm	gm.	gm
Rice-vinegar 1	75	0 003		0.007	
Rice-vinegar 2	75	0 003	0 003	0.006	0.006
Soy bean sauce 1	10	0 022		0.008	
Soy bean sauce 2	10	0.023	0.023	0.008	0 008
Cooking solution 1	25	0.003		0.005	
Cooking solution 2	25	0 003	0 003	0.005	0.005
Cooking solution in recipe	250		0.028		0.048

were estimated on the basis of the calculated weights of calcium and phosphorus in the tricalcium phosphate samples. The percentage of solubility of each element in the cooking solution was decidedly higher than in the water. Taking the highest percentages in all cases, calcium gave 8.09 per cent solubility in water and 30.60 per cent in the cooking solution, whereas the phosphorus showed 6.43 per cent solubility in water and 14.47 per cent in the cooking solution. The results agree with the statement of Cameron and Seidell ('04), that tricalcium phosphate is only slightly soluble in water but more soluble in a dilute acid medium. Comey ('28) found that very small quantities of salts of alkali metals increase the solubility of tricalcium phosphate in water, and that it

is also more soluble when the water contains starch, glue or other animal substances. According to such statements, this cooking solution provides conditions favoring the solubility of tricalcium phosphate. While there was considerable difference in solubility of calcium and phosphorus in samples I and II, however, the cooking in the vinegar-soy bean sauce solution made soluble larger quantities of these elements.

Loss of calcium and phosphorus from bone after cooking in vinegar-soy bean sauce solution. Since it has been shown that the solubility of tricalcium phosphate is greater in acid

TABLE 2
The solubility of $\text{Ca}_3(\text{PO}_4)_2$ in water and cooking solution¹

SOLUTIONS	$\text{Ca}_3(\text{PO}_4)_2$ IN SAMPLE	CALCULATED AMOUNT OF CA IN SAMPLE	CALCULATED AMOUNT OF P IN SAMPLE	AMOUNT OF CA OBTAINED FROM SAMPLE	AMOUNT OF P OBTAINED FROM SAMPLE	PER CENT SOLU- BILITY OF CA	PER CENT SOLU- BILITY OF P
	gm.	gm.	gm.	gm.	gm.		
Water 1	0.842	0.326	0.516	0.026	0.033	8.09	6.43
Water 2	1.603	0.620	0.983	0.027	0.063	4.38	6.39
Cooking solution 1	1.297	0.502	0.795	0.154	0.105	30.60	13.19
Cooking solution 2	1.633	0.632	1.001	0.190	0.145	30.12	14.47

¹ The amounts of Ca and P in the cooking solution were subtracted from the total amounts of these elements obtained from the $\text{Ca}_3(\text{PO}_4)_2$, after it was cooked in the solution.

solution than in water, it may be assumed that there would be larger amounts of calcium and phosphorus dissolved from bone cooked in acid medium than in water. Bone samples were prepared as described. In this particular determination the meat was removed from the bone and weighed before cooking. After the cooking process, all visible pieces of bone were collected for analysis. The results are shown in table 3. The difference in the percentage of calcium and phosphorus in the raw and cooked bone samples might appear to be insignificant but it is constant and a certain loss resulting from cooking may be assumed. From the mathematical calculation, based on the percentages of the calcium and phosphorus in

the raw bone (table 3), a sample of 45.40 gm. of bone should yield 4.354 gm. of calcium and 2.202 gm. of phosphorus. Comparing this theoretical yield with the actual amounts of 3.988 gm. of calcium and 2.058 gm. of phosphorus obtained from the cooked sample, a loss of 0.366 gm. calcium and 0.144 gm. phosphorus is evident.

Calcium and phosphorus in the meat-bone dish. From the above, it seemed possible to interpret the results obtained from the prepared meat-bone dish. Analytical data for the meat and the solution after cooking are shown in tables 4

TABLE 3
Loss of calcium and phosphorus in bone after cooking

BONE SAMPLE	TOTAL MATERIAL	RAW BONE	ASH	CALCIUM	PHOSPHORUS	PER CENT IN RAW BONE	
						Calcium	Phosphorus
			<i>gm</i>	<i>gm</i>	<i>gm.</i>		
Raw 1			5 339	2.043	1.025		
Raw 2			5 072	1.906	0 964		
Total	151.50	41.20	10.411	3.949	1 989	9.59	4.85
Cooked 1			5.271	1 965	1 009		
Cooked 2			5 514	2.023	1 049		
Total	165 30	45 40	10 785	3.988	2.058	8 73	4.53
Theoretical yield from 45.4 gm bone				4.354	2.202		
Loss in 45.4 gm of bone after cooking				0 366	0.144	— 0 81	— 0 32
Loss in 100 gm. of bone after cooking				0.806	0.318		

and 5. The bone samples were also analyzed. Since there was no way to get the separate weight of the raw bone and meat, which were supposed to be cooked together according to the Chinese method, no comparison of the percentage of calcium and phosphorus in these bone samples could be made. During cooking, considerable amounts of the organic materials of the bone dissolved, lessening the net weight of the bone. As a result, a higher percentage of ash in the total weight of the bone is found in the cooked sample than in the raw bone. Therefore, the data of the bone analyses were not included in the interpretation of the yields of calcium and phosphorus from this meat-bone dish. The determinations of

calcium and phosphorus in both raw and cooked meat are found in table 4. From nearly equal amounts of raw material, the meat after it was cooked had an extracted dry weight about two times greater than that of the raw. Consequently, the ash of the cooked meat was about four times greater than the raw, in spite of the fact that considerable amount of the tissue fibers had gone into the solution, as a result of long cooking. The comparison of the amounts of calcium and phosphorus are equally striking. In the cooked meat sample, there were 0.451 gm. of calcium and 0.270 gm. of phosphorus as compared to 0.028 gm. of calcium and 0.098 gm. phosphorus in the raw meat sample. Assuming that the meat from nearly

TABLE 4

Comparison of calcium and phosphorus in meat (raw and after cooked in solution)

SAMPLES	MEAT AND BONE	EXTRACTED MEAT	TOTAL ASH	CALCIUM IN ASH	PHOSPHORUS IN ASH	PER CENT IN ASH	
						Calcium	Phosphorus
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>		
Raw meat 1		6.585	0.253	0.016	0.056	6.44	22.28
Raw meat 2		6.159	0.162	0.012	0.042	7.09	25.74
Total	172.9	12.744	0.415	0.028	0.098	6.69	23.63
Cooked meat 1		13.161	1.131	0.299	0.176	26.43	15.54
Cooked meat 2		9.060	0.556	0.152	0.094	27.30	16.91
Total	173.8	22.221	1.687	0.451	0.270	26.71	13.99

equal weights of the corresponding parts of the same piece of spareribs was equal, a considerable gain of these minerals was obtained as a result of cooking. Since no reported studies could be found regarding the possibility of absorption of calcium and phosphorus by meat during the cooking process, it is assumed that these elements, after being dissolved from the bone must be accounted for in the meat and cooking solution. Another possible assumption could also be made. Due to the salt-forming property of the proteins, calcium may be taken up to form calcium proteinate. The amounts of calcium and phosphorus obtained from the solution after the meat and bone are cooked in it are given in table 5. The calcium in the cooked solution was 0.100 gm. and the phosphorus 0.114

gm., as against 0.028 gm. of calcium and 0.048 gm. of phosphorus in the solution before cooking, thus indicating a gain of 0.072 gm. calcium and 0.066 gm. phosphorus. The reason why the amounts of calcium and phosphorus are not higher is well explained by the previous assumptions that considerable quantities may adhere to the meat or form calcium proteinate.

TABLE 5

Comparison of calcium and phosphorus in solution before and after cooking

SOLUTION	AMOUNT USED	ASH	CALCIUM IN ASH	PHOSPHORUS IN ASH
	cc	gm.	gm.	gm.
First portion after cooking		1.937	0.047	0.050
Second portion		2.334	0.053	0.064
Total	250	4.271	0.100	0.114
Solution before cooking	250		0.028	0.048
Gain after cooking			0.072	0.066

TABLE 6

Distribution of the measurable amounts of calcium and phosphorus in an individual serving of 'sweet-sour-spareribs'

SOURCE	RAW MATERIAL	CALCIUM	PHOSPHORUS	CA P
		gm.	gm.	
Meat and bone	173.8 gm.	0.451	0.270	
Cooked solution	250 cc.	0.100	0.114	
Total		0.551	0.384	1.43

There appears to be sufficient evidence to conclude that in the process of cooking there is a definite loss of calcium and phosphorus from the bone with a relative increase in these minerals in the meat and the cooking solution. The distribution of the measurable amounts of calcium and phosphorus actually obtained from an individual serving of 'sweet-sour-spareribs' is given in table 6. In a 173.8 gm. (about 6.5 ounces) sample of meat and bone, cooked with the diluted vinegar and soy bean sauce solution, according to the method described, 0.551 gm. of calcium and 0.384 gm. of phosphorus are obtained.

DISCUSSION

The increased amounts of calcium and phosphorus in the meat and solution after cooking furnish proof that the increase comes from the bone. Also, the comparative amounts of calcium and phosphorus of the crude bone, raw, and after cooking, give further proof of the loss of these elements from the bone. After cooking, a 45.4-gm. sample of raw bone from 165.3 gm. of spareribs showed a loss of 0.366 gm. calcium and 0.144 gm. phosphorus. From a slightly larger amount of spareribs, namely 173.8 gm., it may be assumed that the weight of the raw bone would be somewhat higher and, therefore, the total loss of these elements would be greater. The loss can be accounted for when we compare the gain of calcium and phosphorus in the meat and solution after cooking (tables 4 and 5).

It thus appears from the data on the meat and solution after cooking that this one individual serving of 'sweet-sour-spareribs' composed of 173.8 gm. of bone and meat with 250 cc. of the vinegar and soy bean sauce solution, cooked at low temperature for 1 hour, furnished 0.551 gm. of calcium and 0.384 gm. of phosphorus. These amounts gave a Ca:P ratio of 1.43. Although the amounts of calcium and phosphorus obtained give a ratio believed to be favorable for assimilation (Daniels and Hutton, '28), the forms in which those elements exist and the physiological availability for human utilization require further experimental study. According to Sherman ('32) the daily allowance for the average man is 0.68 gm. of calcium and 1.32 gm. of phosphorus. When these elements are adequately furnished, the forms and ratio in which they are supplied are of less importance.

If these amounts of 0.551 gm. of calcium and 0.384 gm. phosphorus obtained from this 'sweet-sour-spareribs' dish were assimilated as satisfactorily as the well-recognized sources, such as milk and cheese, this special way of cooking meat and bone is one which should be favored and used plentifully in the diet, especially when milk is not provided in adequate amounts.

SUMMARY AND CONCLUSION

1. In the Chinese diet, milk and cheese, the well-recognized sources of calcium and phosphorus, are not used. A typical Chinese dish, which is called 'sweet-sour-spareribs,' was analyzed as a possible source of these elements. Pork spare-ribs were cut into 1½-inch pieces and cooked in a rice-vinegar, soy bean sauce, salt and sugar solution for 1 hour at a low temperature. Samples of bone, meat and cooking solution were analyzed for calcium and phosphorus before and after cooking.

2. Tricalcium phosphate is found to be more soluble in the cooking solution with a pH of 3.2 containing salt and sugar, than in water. Providing the calcium and phosphorus of bone exist in large proportion as tricalcium phosphate, this cooking solution would favor its solubility.

3. From the analysis of a sample of 173.8 gm. of meat and bone, total amounts of 0.551 gm. of calcium and 0.384 gm. of phosphorus were obtained. Of these amounts, 0.451 gm. calcium and 0.270 gm. phosphorus were obtained from the meat, and 0.100 gm. calcium and 0.114 gm. phosphorus from the solution, after cooking. In this particular brand of soy bean sauce, only traces of calcium and phosphorus were found, while those of the rice-vinegar were negligible.

4. Quantitatively, the amount of calcium obtained in this dish exceeds the minimum requirement of 0.45 gm., and approaches the allowance of 0.68 gm. per man per day, while the amount of phosphorus obtained hardly reaches half the minimum requirement. The latter, however, is more generously distributed in foods and it is easier, therefore, to fulfill the daily requirement. Biological experiments should be carried on in animals and humans to find out how these elements from such a source are metabolized. If they are absorbed and utilized satisfactorily, another significant source of calcium and phosphorus has been demonstrated.

5. It is possible that this peculiar method of cookery used extensively by the Chinese may be of particular value in providing adequate amounts of calcium and phosphorus in the Chinese diet. Also the Chinese method of chewing and sucking small pieces of bones while eating may increase the total amounts of these elements.

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HYPERTHYROIDISM AND NUTRITION

I. VITAMIN B AND THYROXIN¹

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EIGHT FIGURES

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The protective influence of various foodstuffs in experimental hyperthyroidism (rat) has been investigated during the last 10 years by Abelin and associates ('30) who found that the basic metabolic rate produced by excessive amounts of desiccated thyroid or thyroxin can be materially reduced and liver injury prevented to a great extent by feeding large amounts of pure casein, yeast and egg yolk, and by certain glandular organs, such as kidney.

That the vitamin B requirement of the animal organism is determined chiefly by its calorific requirement has been pointed out in 1927 by Plimmer, Rosedale and Raymond ('27) and by Cowgill and Klotz ('27).

Himwich, Goldfarb and Cowgill ('31) reported an increase in the requirement of undifferentiated vitamin B of the dog during intervals of feeding desiccated thyroid, as determined by the period of onset of anorexia and loss of body weight. Recently Cowgill and Palmieri ('33), using desiccated thyroid and Harris yeast as a source of vitamin B, found similar results with pigeons, that is, the vitamin B requirement proved to be greater in hyperthyroidism than under 'normal' conditions.

¹ Research paper no. 302, Journal series, University of Arkansas.

Recently von Euler and Klussmann ('32) observed that rats on a diet deficient in vitamin A and injected with thyroxin suffered loss of weight less rapidly when also receiving a daily dose of carotin (or carotene).

EXPERIMENTAL

In this investigation a study was made of the toxicity of thyroxin in the albino rat and of the protective influence of the vitamin B complex and vitamin B against the injury produced by thyroxin. A highly concentrated vitamin B extract was used, prepared by one of us (B. S.), which is 20,000 times as potent in vitamin B as cow's milk (Sure, '32). Three to 5 mg. of the extract, when given orally daily, resulted in a growth of 12 to 15 gm. weekly per animal. The thyroxin employed was a pure crystalline product secured from E. R. Squibb & Sons.

The results of our findings are summarized in charts 1 to 8 inclusive. In all this work litter mates of the same sex were employed.

Toxicity of thyroxin on diets abundant and deficient in the vitamin B complex

It is apparent from charts 1 and 2 that the albino rat can tolerate large amounts of thyroxin when taken in by mouth, ♂ 9638 having survived 19 days and ♂ 9635 a period of 36 days on as much as 1 mg. daily. Male no. 9637, however, survived only 5 days on the same daily dosage, but this animal was probably already injured by cumulative effects of the smaller dosages which it received during the previous 3 weeks.

From charts 3 and 4, it will be noted that 0.01 to 0.02 mg. thyroxin given daily by mouth produced no deleterious effect on the rat as judged by changes in body weight, on a ration (no. 1845) abundant in the vitamin B complex, of the following composition: casein (purified), 20; salts no. 185, 4 (McCollum and Simmonds, '18); Northwestern yeast (baker's, dehydrated), 10; dextrin, 56.

It is evident from chart 3 that on ration 1845 no decline in weight has occurred following subcutaneous injections of 0.25 mg. thyroxin daily for a period of 12 days. When, however,

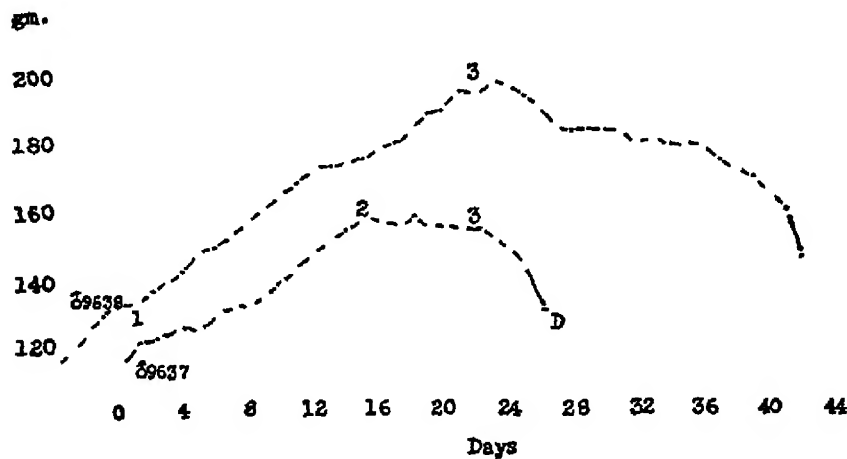


Chart 1, showing toxicity of thyroxin administered orally, supplementing a ration abundant in the vitamin B complex.

1. 0.1 mg thyroxin daily.
2. 0.2 mg. thyroxin daily.
3. 1.0 mg thyroxin daily.
- D=Died

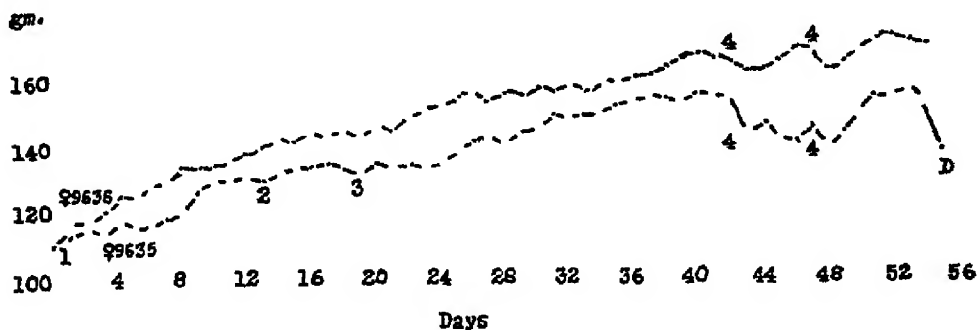


Chart 2, showing toxicity of thyroxin administered orally, supplementing a ration abundant in the vitamin B complex

1. 0.1 mg. thyroxin daily.
2. 0.2 mg. thyroxin daily.
3. 1.0 mg. thyroxin daily.
4. Fasted 18 hours previous to sampling for blood cholesterol.
- D=Died

the ration was changed to one deficient in the vitamin B complex (no. 1751), ♀ 9614 that received no thyroxin survived with a loss of 30 gm., while its litter mate died on the thirteenth day of the vitamin depletion period, with a loss of 50 gm. of body weight.

Chart 4 shows the toxic effect of 0.25 mg. thyroxin administered subcutaneously six times a week. Male no. 9615 stopped growing on ration 1865 as soon as the parenteral injections of thyroxin were begun and survived 23 days. Its

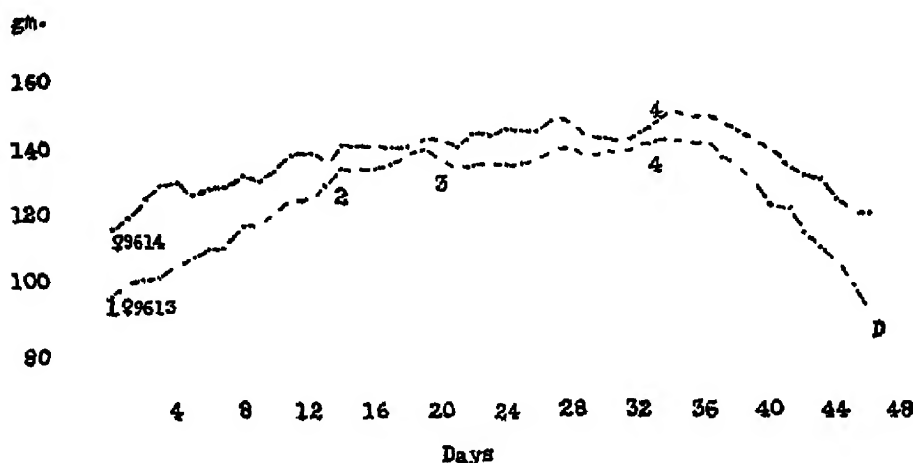


Chart 3, showing toxicity of thyroxin administered subcutaneously, on a ration abundant, which was later changed to one deficient in the vitamin B complex

1. 0.01 mg. thyroxin daily, orally.
 2. 0.02 mg. thyroxin daily, orally.
 3. 0.25 mg. thyroxin subcutaneously daily, with the exception of Sundays.
 4. Ration changed so that it was deficient in the vitamin B complex.
- D=Died

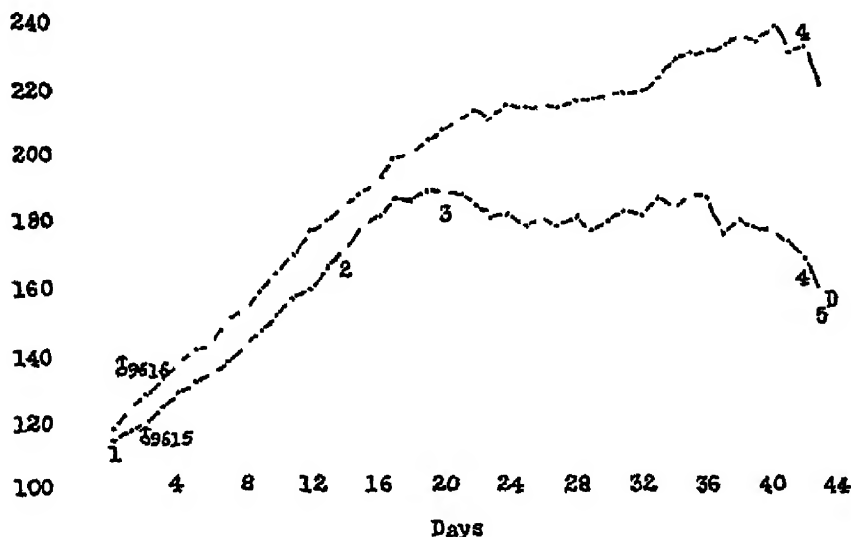


Chart 4, showing toxicity of thyroxin administered subcutaneously, supplementing a ration abundant in the vitamin B complex.

1. 0.01 mg. thyroxin daily, orally
2. 0.02 mg thyroxin daily, orally.
3. 0.25 mg. thyroxin subcutaneously daily, with the exception of Sundays.
4. Fasted 18 hours previous to sampling for blood cholesterol.
- 5 Died while obtaining blood sample peripherally.

litter mate, ♂ 9616 served as a control, which made continuous growth on the same ration, without thyroxin administration.

From chart 5 it is apparent that as much as 0.25 mg. thyroxin administered subcutaneously for 10 days had no noteworthy catabolic effect on ♀ 9617, since there were no losses in body weight. When, however, the diet of this animal as well as that of its litter mate was depleted of the vitamin B complex, striking differences in results were obtained. Female no. 9617, that received the thyroxin, died in 11 days, accompanied by a loss of 47 gm. in body weight, while its litter

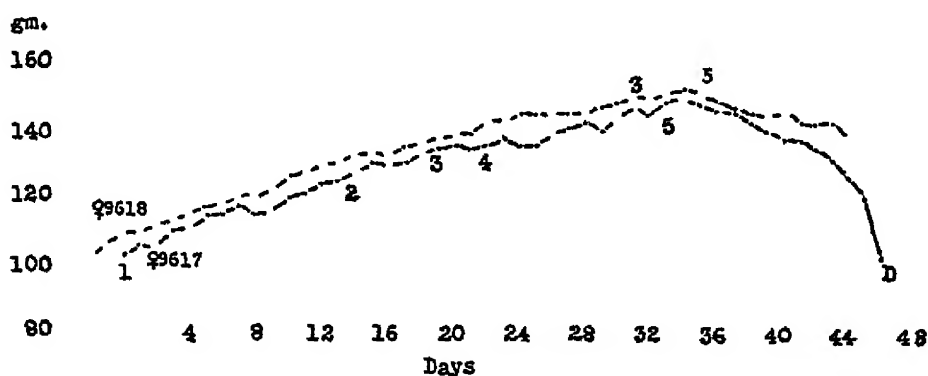


Chart 5, showing toxicity of thyroxin administered subcutaneously, on a ration abundant, which was later changed to one deficient in the vitamin B complex

- 1 0.02 mg. thyroxin daily, orally.
 2. 0.04 mg. thyroxin daily, orally.
 3. Thyroxin removed.
 4. 0.5 mg. thyroxin subcutaneously daily, with the exception of Sundays.
 5. Ration changed so that it was deficient in the vitamin B complex.
- D=Died

mate, ♀ 9618, that received no thyroxin, survived, with a loss of only 11 gm.

Male no. 9619 (chart 6) survived for 23 days, following injection of as high as 0.5 mg. thyroxin daily, six times a week, on our ration 1865, containing an abundance of vitamins B and G in the form of dried baker's yeast.

Protective influence of the vitamin B complex against toxicity of thyroxin

It is evident from chart 7 that a daily oral administration of 0.08 to 0.16 mg. thyroxin for a period of 19 days apparently produced a depressing effect on the growth of ♂ 9634, since

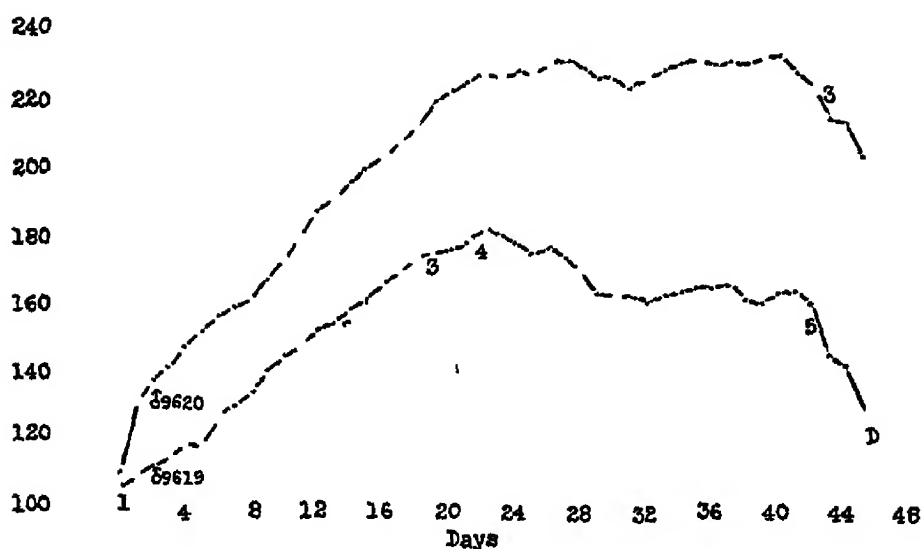


Chart 6, showing toxicity of thyroxin administered subcutaneously, supplementing a ration abundant in the vitamin B complex.

1. 0.02 mg. thyroxin daily, orally.
 2. 0.04 mg. thyroxin daily, orally.
 3. Thyroxin removed
 4. 0.5 mg. thyroxin subcutaneously daily, with the exception of Sundays.
 5. Fasted for 18 hours previous to sampling blood for cholesterol.
- D=Died

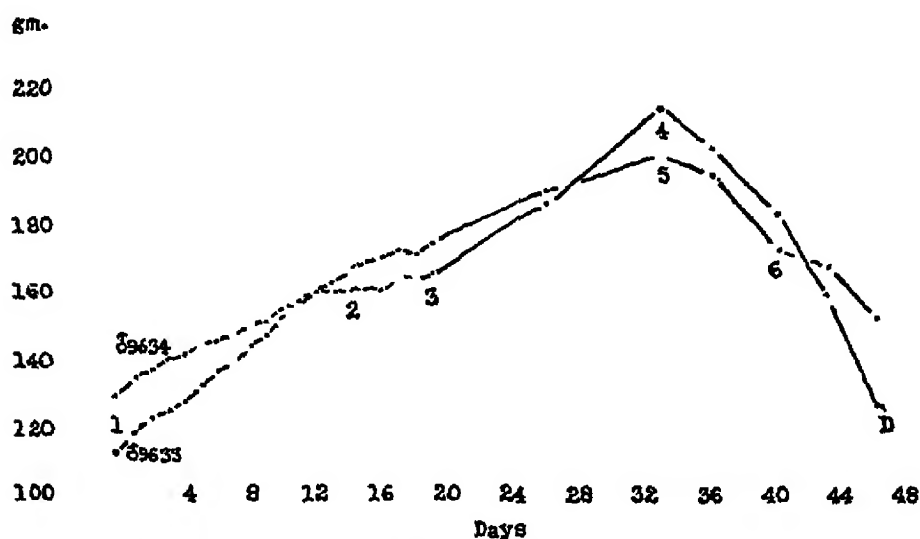


Chart 7, showing the protective influence of the vitamin B complex against the toxicity of thyroxin.

1. 0.08 mg thyroxin daily, orally
 2. 0.16 mg. thyroxin daily, orally
 3. Thyroxin removed.
 4. 1.0 mg. thyroxin daily, orally, and ration changed so that it was deficient in the vitamin B complex.
 5. 1.0 mg. thyroxin daily, orally, ration depleted of the vitamin B complex, and 12 mg. of vitamin B concentrate administered daily separately from the ration.
 6. Vitamin B concentrate increased to 24 mg. daily.
- D=Died

the removal of thyroxin during the subsequent 14 days was followed by a much more marked rate of growth. The noteworthy fact, however, in this experiment became apparent when a daily oral dose of 1 mg. was begun on the thirty-third day of the experiment. This daily dose was administered to both animals 9633 and 9634. At the same time the ration was depleted of the vitamin B complex. Male no. 9634, however, was given separately from the ration 12 mg. of a highly concentrated vitamin B preparation, which was increased 7 days later to 24 mg. Male no. 9633 died on the thirteenth day of the vitamin depletion period with a total loss of 110 gm., or 52 per cent of its body weight, while ♂ 9634, which was placed on the same ration depleted of the vitamin B complex, and which received the same daily dose of thyroxin, but because it received a vitamin B concentrate during this experimental period, survived with a loss of only 47 gm., or 23.5 per cent of its body weight.

Protective influence of vitamin B against toxicity of thyroxin

Chart 8 indicates that a daily oral dose of 0.03 to 0.06 mg. thyroxin for 21 days produced no noteworthy depressing effect on the growth of ♀ 9623. A daily subcutaneous dose of 0.25 mg.,² however, on a ration (no. 2345) deficient only in vitamin B³ (Sure, '33) was followed by a total collapse of the animal in 30 days, accompanied by a loss of 98 gm., or 54 per cent loss of body weight; while litter mate, ♀ 9624 that was subjected to the same treatment, but because it received during the experimental period 6 to 18 mg. daily of a highly potent vitamin B concentrate, survived with a loss of only 26 gm., or 16 per cent loss of body weight.

The several blood cholesterol determinations made on pathological and control animals indicate a hypocholesteremia during periods of hyperthyroidism. This point we do not consider, however, as yet established until we have corroborated such findings on a large number of animals.

— Daily with the exception of Sundays.

² Composition of ration 2345 is as follows: Casem (purified), 10, autoclaved round beef steak, 15, salts no. 185, 4; butter fat, 10; dextrin, 61.

Necropsy findings of animals that succumbed from hyperthyroidism showed slight thoracic hemorrhages, peripheral and venous constriction and arterial dilatation. The heart and blood vessels, particularly veins, were filled with blood. The

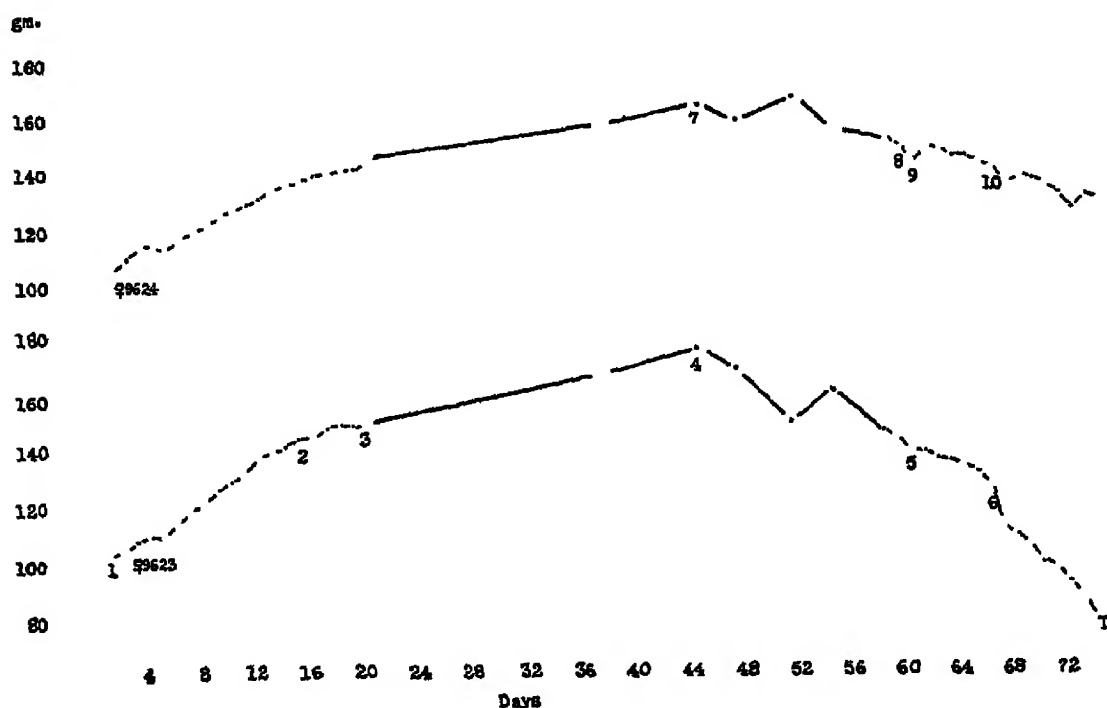


Chart 8, showing the protective influence of vitamin B against the toxicity of thyroxin.

1. 0.03 mg. thyroxin daily, orally
2. 0.06 mg. thyroxin daily, orally.
3. Thyroxin removed.
4. 0.5 mg. thyroxin subcutaneously daily, with the exception of Sundays, and ration depleted of vitamin B.
5. Bled peripherally for blood cholesterol.
6. Fasted for 18 hours previous to sampling for blood cholesterol.
- D = died.
7. 0.5 mg. thyroxin subcutaneously daily, with the exception of Sundays, ration depleted of vitamin B, and 6 mg. of vitamin B concentrate daily administered separately from the ration.
8. Increased vitamin B concentrate to 18 mg. daily.
9. Bled peripherally for blood cholesterol.
10. Fasted for 18 hours previous to sampling for blood cholesterol.

heart appeared hypertrophied and fibrous on macroscopic examination. In one case the thyroid was enlarged.

Since excessive amounts of thyroxin stimulate catabolism of body tissues and particularly injury of the liver (Abelin,

'30, Abelin, Knochel and Spichtin, '30) which is the storage depot of vitamin B, it is of course to be expected that during the process of tissue oxidation the vitamin B will be at the same time destroyed; hence, the greater requirement of this vitamin the more thyroxin is made available in circulation.

A study of a quantitative balance between amount of thyroxin and vitamin B concentrate is in progress, the results of which will be reported later.

The fact that in experimental hyperthyroidism protection can be afforded by a highly concentrated vitamin B preparation, would suggest that oral or preferably parenteral administrations of potent standardized vitamin B concentrate may be indicated in toxic goiter, particularly in non-operative cases. This is, however, a problem for the clinician to solve.

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THE STORAGE OF VITAMIN A IN THE LIVER OF THE RAT¹

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ONE FIGURE

(Received for publication July 25, 1933)

INTRODUCTION

In the course of a prolonged study of the effects of isolated radiations of equal energy in the visible and infra-red regions of the spectrum upon the growth and development of normal rats, the following results, though in the nature of a side issue to the main problem, seem to merit publication.

In such a study, our method has been to raise rats on a standard normal diet, and to vary nothing in the experiment, except the isolated band of radiation to which the rat has been exposed. The diet chosen for this purpose was the well-known Sherman B diet (Sherman and Campbell, '24), which consists of dried whole milk one-third, ground whole wheat two-thirds, NaCl 2 per cent of the wheat. In the course of our experiments we observed at autopsy the occurrence of spontaneous middle ear infections occurring in a small percentage of our older rats. This infection did not appear to interfere with the general health of the animals, but we had evidence that it produced a slight limitation of growth as compared with the growth of non-infected animals. In view of the many claims for the anti-infective nature of vitamin A we con-

¹Aided by a grant from the Committee on the Effects of Radiation upon Living Organisms, Divisions of Biology and Agriculture, National Research Council

sidered the adequacy of the vitamin A content of the diet and decided to study it by chemical methods. In this diet the supply of vitamin A comes mainly from dried whole milk, whole wheat being a poor source of this factor. Milk is known to be a variable source of vitamin A, its potency in this factor depending upon the diet of the cow, and being subject to seasonal variations. In addition to this, the possibility of the destruction of vitamin A during the process of evaporation would tend to decrease its value.

We decided to make chemical determination of the vitamin A content of the blood, livers, and body fat of a series of our animals, with and without the addition of vitamin A to the diet.

Rosenheim and Webster ('27) observed that halibut liver oil was a much more potent source of vitamin A than cod liver oil. This observation has since been abundantly confirmed. Halibut liver oil has the additional advantage of containing very little vitamin D, so for these reasons we used it as a source of additional vitamin A in our experiments. The rats used in these experiments were of The Wistar Institute Experimental Colony strain. The mothers were shipped to us during the latter part of their pregnancy. The young were reared after weaning on the Sherman B diet.

Technic employed for the collection of samples

Blood. The rat was anaesthetized with illuminating gas. It was then bled rapidly from the heart, and the blood collected into a small test tube of 10 ml. capacity. When clotting had occurred the serum was analyzed for vitamin A by the method described below.

Liver. The abdomen was opened and the entire liver carefully removed. After this organ had been freed from adherent tissue, and any excess of moisture or blood removed with clean filter paper, it was weighed accurately to 0.01 gm. It was then analyzed for vitamin A by the method described below.

Body fat. Both perinephric and mesenteric fat were taken from the rat, and a mixed sample of these used in making the tests.

METHOD

Reagents

With the exception of the alcohol, which was the pure undenatured 95 per cent alcohol obtained from the American Commercial Alcohol Company, all the chemicals described below were Baker's C.P. analyzed quality.

Chloroform. It is essential that the chloroform used should be as nearly anhydrous as possible and free from alcohol. To insure this Baker's C.P. chloroform was thoroughly washed with a large excess of distilled water, dried with anhydrous calcium chloride, and distilled, by means of a Clarke's fractionating column (Clarke and Rahrs, '23) over anhydrous calcium chloride.

Antimony trichloride reagent. To approximately 300 cc. of the anhydrous chloroform, 100 gm. of antimony trichloride were added. After the chloroform was well saturated the excess of the salt was filtered off, and the clear solution stored in a tightly stoppered brown glass bottle.

Copper sulfate standard solution. Ten grams of copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) were dissolved in 90 cc. of distilled water.

Fifty per cent ethyl alcohol. To 500 cc. of the 95 per cent ethyl alcohol 500 cc. of distilled water were added.

Determination of vitamin A

Blood. The tube containing the clotted blood was centrifuged. Two cubic centimeters of clear serum were placed in a special tube with a narrow neck of 10 ml. capacity, 2 cc. of 95 per cent ethyl alcohol added, and the contents well mixed; finally 2 cc. of petroleum ether were introduced and the tube was shaken at intervals for 10 minutes. It was then centrifuged and 1 cc. of the clear layer of petroleum ether transferred to a 10 ml. test tube. This tube was placed in a beaker

containing water at 40 to 45°C., the petroleum ether was rapidly evaporated by the introduction of a gentle current of dry air into the tube. The residue was dissolved in 0.1 cc. of the anhydrous chloroform, 1 cc. of the antimony trichloride reagent was added, and the intensity of the resulting blue color determined 30 seconds after mixing in a colorimeter, using the copper sulfate solution as a standard. By definition, 1 arbitrary unit of vitamin A is said to be present when standard and unknown match exactly. If, therefore, the standard and unknown match in the case of serum or plasma, the vitamin A units of the sample will be 100 units per 100 cc. This unit is approximately 0.01 Sherman unit.

Liver. One gram of liver (weighed to 0.01 gm.) was transferred to a small mortar, fine sharp sand was added and 5 cc. of the 50 per cent ethyl alcohol. After thorough grinding, the mixture was poured into a 50 cc. centrifuge tube with a narrow neck. Three additional 5 cc. portions of 50 per cent alcohol were added successively to the mortar, ground, and rinsed into the tube, so that the tube contained all the liver to be analyzed. Finally, 20 cc. of petroleum ether were added to the tube; the tube was stoppered and shaken at intervals for 10 minutes. The tube was then centrifuged and the vitamin A determined in the petroleum ether layer by the method described for blood. It is desirable to use such an amount of the petroleum ether extract that the standard in the colorimeter will read between 20 and 30 mm. with the unknown set at 20 mm. The average of three determinations on separate portions of the extract was taken in making the final calculation, which is expressed as the number of blue units per 100 gm. of liver.

We found that the blood and liver of rats fed on the Sherman B diet did not contain significant amounts of the carotinoid pigments. Therefore no corrections for the presence of these pigments were made in the values obtained.

Body fat. The method employed was the same as that described for liver. It was found that the presence of too much fat interfered with the color given in the antimony trichloride

reaction, therefore no more than 2 cc. of the petroleum ether extract was used in preparing the solution for each colorimetric reading.

The above method is that of Carr and Price ('26) with some modifications by S. W. Clausen as yet unpublished.²

Experiment 1. Young rats

Six rats, 54 days old were used. Three of them were fed 1 drop of halibut liver oil (Abbott Laboratories, serial no. 25954). The remaining three kept as controls were fed no oil. They were killed 24 hours later and the livers, blood and body fat analyzed for vitamin A. This experiment was a preliminary one, and it should be mentioned that the rats used came from three families that after weaning had developed a spontaneous infection with *Pasteurella*, which, since it was entirely limited to this group, was probably acquired during transportation. Various other members of these families had died, bacteriological examinations had been made by Prof. George Berry, and we had decided to sacrifice the whole group to prevent spread of infection. The six rats used showed some slight loss of weight at the time the experiment was done, and may have been infected with *Pasteurella* at the time the oil was given. We have no reason to think that in such a short experiment this possible intercurrent infection had any influence on the results.

²“The modifications as described above are. a) the use of a relatively small volume of blood, 2 cc. serum, as compared with 10 to 20 cc. blood used by van Eskelen ('31) and Menken ('32); b) the carrying out of the blood extraction in one step as compared with the methods described by van den Bergh and Muller ('20); c) the selection of 10 per cent copper sulfate as a standard for colorimetric use instead of a Lovibond Tintometer; d) the rapid extraction of vitamin A from liver and fat by simultaneous use of two immiscible solvents, as compared with the more prolonged procedures described by Wilson ('27), Simmonet et al. ('31) and Moore ('30). The dilute alcohol penetrates the tissue, and liberates the vitamin A, which is immediately passed on into the much better solvent, petroleum ether. The latter solvent alone does not readily extract vitamin A or carotinoids from moist tissues. This method has proved to be simple and rapid, and it avoids the danger of the destruction of vitamin A by too vigorous treatment. In criticism of the method it may be said that the blue color is not absolutely characteristic of vitamin A, or altogether proportional to the amount present, because the fatty acids have not been removed by saponification. Whilst fully alive to this criticism, we found such wide differences in our results under varying conditions of experimentation, and such good agreement in the results of the groups treated alike, that we feel the results justify the use of the method.” (S.W.C.)

The results are seen in table 1. The oil was given from a dropper in a dark room by the light of a 10 watt green Mazda lamp, and one rat, no. 93, was fed 2 drops in error. It is interesting to note the increased vitamin A in the blood, liver and body fat of this rat. None of these rats showed any infection of the middle ear. In this group the whole livers were not weighed at autopsy; the calculations for vitamin A were based on the amount per 100 gram liver. In order to bring this series into line with our other experiments, where the livers were weighed, we calculated the vitamin A in the whole livers on the basis of the liver weights according to body weight given by Donaldson ('24).

TABLE 1
Young rats, Sherman B diet 54 days old

TREATMENT	NO RAT	LITTER	SEX	LIVER			BLOOD UNITS VITAMIN A/100 CC.	BODY FAT UNITS VITAMIN A/100 GM	PUS IN EARS
				Weight, grams ¹	Units vitamin A/100 gm.	Units vitamin A whole (calcu- lated)			
No added oil	89	XVI	♂	4.50	310	13.9	16	0	—
	90	XVI	♂	4.50	325	14.6	14	0	—
	91	XVI	♀	4.50	275	12.4	14	0	—
Average				4.50	303	13.6	14.5	0	
1 drop hali- but liver oil 24 hours be- fore death	92	XVI	♀	4.00	2290	91.6	18	63	—
	93 ²	XVII	♂	5.00	6220	301.0	34	138	—
	94	XVII	♂	4.50	2910	130.9	21	79	—
Average nos. 92, 94				4.25	2600	111.2	19.5	71	

¹ Liver weights taken from Donaldson's graph.

² Fed 2 drops in error.

Experiment 2. Adult rats

Three litters of rats, twenty-four rats in all, which had been reared on the Sherman B diet, were used. At the age of 174 days they were divided into two groups, carefully selected so that the same litters and sexes were represented in both groups. One group of eleven rats was fed 1 drop of halibut liver oil every week for 13 weeks. The remaining thirteen rats were kept as controls with no addition to the diet. At the age of 264 days these rats were killed and analyses of liver and blood for vitamin A were made. In this experiment the animals were killed 6 days after the administration of the last dose of halibut liver oil. Results are seen in table 2.

Experiment 3. Adult rats, further observations

Five groups of adult rats, which varied in age from 186 to 204 days were fed different doses of halibut liver oil at weekly intervals as in experiment 2, 1 drop per week being fed. These

TABLE 2
Adult rats, Sherman B diet 264 days old

TREATMENT	NO. RAT	LITTER	SEX	LIVER			BLOOD UNITS VITAMIN A/100 CC.	BODY FAT UNITS VITAMIN A/100 GM.	PUS IN EARS
				Weight, grams	Units vitamin A/100 gm.	Units vitamin A whole			
No added oil	69	XIV	♂	8.06	2,180	176	14	15	—
	71	XIV	♂	7.71	2,957	228	10	60	+
	72	XV	♀	5.42	2,940	159	24	25	++
	74	XV	♂	8.36	1,225	102	12	10	—
	81	XIV	♀	5.33	2,630	140	7	20	—
	83	XIV	♀	6.22	5,175	322	14	35	++
	85	XV	♀	5.20	5,625	293	11	65	—
	86	XV	♂	8.30	490	41	18	40	—
	88	XV	♀	6.30	4,880	308	13	25	—
	65	XIII	♀	5.69	2,070	118	102	70	—
	67	XIII	♀	6.39	3,660	234	16	0	—
	76	XIII	♂	8.48	835	71	26	40	+
	79	XIII	♀	5.62	2,280	128	13	80	—
Average				6.70	2,820	178	14	37	
1 drop hali- but liver oil weekly for 13 weeks	70	XIV	♀	5.08	48,500	2462	19	111	+
	73	XV	♀	6.43	26,000	1672	15	115	++
	75	XV	♀	6.25	37,167	2334	10	160	—
	80	XIV	♀	6.27	38,000	2383	8	85	—
	82	XIV	♀	5.75	35,833	2060	20	110	—
	84	XV	♂	7.20	32,667	2354	13	...	++
	87	XV	♂	9.35	24,500	2290	24	...	—
	66	XIII	♂	7.34	19,583	1437	21	140	—
	68	XIII	♂	7.73	34,000	2627	25	65	—
	77	XIII	♀	5.59	31,667	1771	19	180	—
	78	XIII	♀	5.88	37,333	2196	14	95	+
Average				6.62	33,204	2144	17	118	

Nos. 70 to 78 last drop of oil given 6 days before death.

five groups received a total of 4, 6, 7, 9 and 11 drops, respectively. They were killed at the widely differing intervals of 28, 137, 132, 97 and 64 days after the administration of the last drop of oil. The object of this experiment was to trace, if possible, additional storage in the liver with increased

dosage, and to find out evidence, if any, of a liberation of vitamin A from the liver in adult life. Results are seen in table 3 and figure 1. The vitamin A values in the liver, already given in table 2, are included in table 3 and figure 1 to complete the story.

Experiment 4

A family of seven young rats, whose mother had received 1 drop of halibut liver oil the day before the young were born, and 1 drop per week during the 3 weeks of lactation, were killed at the age of 54 days and analyses for vitamin A in tissues made. Results of this experiment are seen in table 4.

TABLE 3
Adult rats, Sherman B diet

AGE WHEN KILLED (DAYS)	NUMBER OF RATS	TOTAL NUMBER OF DROPS FED	KILLED DAYS AFTER LAST DROP	LIVER—AVERAGE			BLOOD AVERAGE UNITS VITAMIN A/100 CC.	BODY FAT AVERAGE UNITS VITAMIN A/100 G.M.
				Weight, grams	Units vitamin A /100 gm.	Units vitamin A whole		
264	13	None	...	6.70	2,820	178	14	37
186	3	4	28	6.98	13,517	946	10	57
203	3	6	137	4.30	27,233	1170	12	23
206	3	7	133	5.48	23,800	1334	13	13
204	5	9	97	4.53	36,423	1619	13	78
200	11	11	64	7.86	21,950	1712	23	147
264	11	13	6	6.62	33,204	2144	17	118

Experiment 5

One drop of the oil used was found by our method of analysis to contain 437 units of vitamin A. We found that measurement by drops from the dropper supplied with the halibut liver oil bottle was accurate to 2 per cent; 1 cc. of the oil being equal to 51 drops. It seemed advisable to determine how much of a large dose could be recovered from the body of rats in terms of units of vitamin A, and also its distribution throughout the body tissues of the rat. Five mother rats were taken, all of whom had been fed a total of 6 drops of halibut liver oil during pregnancy and lactation, whose tissues, therefore, might be considered to be well stored. They were killed approximately 6 weeks after the birth of their young. Three of them were fed no extra halibut liver oil, the other two were fed 4 drops, in one dose, 24 hours before death. Analyses were made of the tissues listed in table 5. The values given are calculated in each case on the basis of the

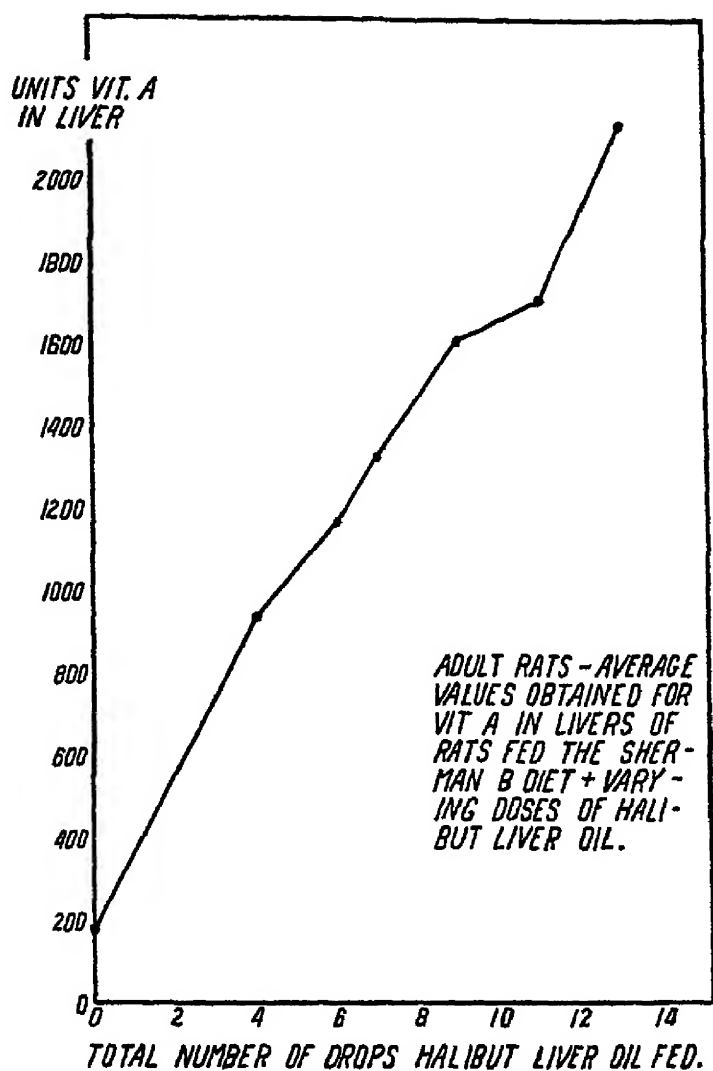


Figure 1

TABLE 4

Young rats, Sherman B diet 54 days old

TREATMENT	NO RAT	LITTER	SEX	LIVER			BLOOD UNITS VITAMIN A/100 CC	BODY FAT UNITS VITAMIN A/100 GM	PUS IN EARS
				Weight, grams	Units vitamin A/100 gm	Units vitamin A whole			
Mother fed a total of 4 drops halibut liver oil. 1 drop the day before birth of young, 1 drop weekly during lactation	142	XXII	♂	4.90	560	27.6	23	5	—
	143	XXII	♀	3.61	615	22.2	16	41	—
	144	XXII	♀	3.45	615	21.2	19	16	—
	145	XXII	♂	4.97	485	24.1	18	14	—
	146	XXII	♂	4.93	482	23.7	18	17	—
	147	XXII	♂	4.91	484	23.8	14	10	—
	148	XXII	♀	3.97	567	22.6	19	14	—
Average				4.40	544	23.5	18	16.7	

weight of the whole organ as weighed at autopsy, with the exception of fat, skeletal muscle and bone. In estimating the total amount of these tissues the figures given by Donaldson were used; viz., 8 gm. for fat, 22 gm. for muscle, and 12 gm. for bone. These are approximate figures for the age and size of the rats. The total amount of blood serum was estimated roughly at 9 cc. The amounts of vitamin A present in these four tissues were so small as to have very little effect on the final result. Therefore we feel that this somewhat rough calculation of the amount of tissue present is justified. In general the technic for analyzing these tissues was similar to that described for liver and fat. For analyses of skin and bone the tissues were frozen with a mixture of acetone and

TABLE 5

Analysis of tissues and organs of female adult rats, for vitamin A content—with and without addition of halibut liver oil, 24 hours before death

RAT NO.	TREATMENT	LIVER UNITS	BLOOD UNITS	FAT UNITS	KIDNEYS UNITS	ADRENALS UNITS	PANCREAS UNITS	SPLEEN UNITS	THYROID UNITS
XXIII	No extra H. O	1453	1.7	3.8	1.24	0.58	0.01	0.20	0
XXVI		1788	0.9	4.0	0.51	0.91	0.08	0	0.02
XXVIII		1393	1.4	3.1	0.85	0.70	0.09	0.05	0
Average		1545	1.3	3.6	0.87	0.73	0.06	0.08	0.007
XXIV	4 drops H.O. 24 hours be- fore death	2977	2.7	6.4	1.40	2.10	0.25	0.30	0.05
XXVII		2563	1.8	5.8	2.20	3.30	0.13	0.10	0.02
Average		2770	2.3	6.1	1.80	2.70	0.19	0.20	0.035

		MUSCLE (SKELETAL) UNITS	LUNGS UNITS	HEART UNITS	BRAIN UNITS	SKIN UNITS	BONE UNITS	INTESTINE UNITS	OVARIES UNITS
XXIII	No extra H. O.	4.8	0.90	0.03	0	9.60	2.6	1.90	0.01
XXVI		1.1	1.02	0.03	0	8.81	3.6	2.23	0.03
XXVIII		8.1	2.21	0.05	0.05	4.27	2.6	0	0.08
Average		4.7	1.38	0.04	0.02	7.56	2.9	1.38	0.04
XXIV	4 drops H. O. 24 hours be- fore death	11.0	10.80	0.40	0.43	18.90	2.4	40.43	0.14
XXVII		10.7	12.30	0.18	0.09	18.97	4.4	132.99	0.13
Average		10.8	11.55	0.29	0.26	18.93	3.4	86.71	0.14

Average total units, nos. XXIII, XXVI and XXVIII = 1570

Average total units, nos. XXIV and XXVII = 2915

Total number of units fed, nos. XXIV and XXVII = 1748

Units recovered = 1345

Per cent recovery = 77

solid carbon dioxide in a mortar, a volume of water equal to that of the acetone added, and the mixture extracted with petroleum ether, after this the general technic described for the other tissues was followed. Results of this experiment are seen in table 5.

DISCUSSION OF RESULTS

A comparison of the control rats in tables 1 and 2, which received the Sherman B diet only, shows a constant average level of vitamin A in the blood at 54 days old and at 264 days old. The average value for three rats at 54 days being 14.5 units per 100 cc. and that for thirteen rats at 264 days being 14 units per 100 cc. It is also clear from these control animals that vitamin A is stored with age in the body fat and in the liver. At 54 days no vitamin A was found in the body fat, at 264 days an average value of 37 units per 100 gm. was obtained. At 54 days the average value of vitamin A per 100 gm. liver was 303 units, at 264 days 2820 units. This storage of vitamin A in liver and body tissues, and its constant level in the blood, would indicate that the animal is able to obtain from the diet some vitamin A for storage in adult life. There is still, however, a possibility that the initial requirements in infancy have not been fully met, since the fuller storage of the liver with vitamin A, and its appearance in the body fat, occurs later.

Sherman B diet + added vitamin A. The immediate storage, after 24 hours, of the liver with vitamin A, its appearance in the body fat, and a raised content in the blood is very striking (table 1), especially when we consider rat no. 93 where a double dose of the oil resulted at once in about twice the amount of vitamin A in blood and fat, and more than twice the amount in liver per 100 gm. compared with the values for rats nos. 92 and 94. In the adult rats (table 2) this fact of storage is abundantly confirmed. In this experiment the last dose of oil was given 6 days before the animals were killed. It is interesting to note that the level of vitamin A in the blood of these two groups, with and without the addition of vitamin A is not significantly different. The average values

of the group receiving oil were slightly higher, but the range of values in the two groups was the same. The values for the blood of those receiving oil give no indication of the enormous storage in the liver. This suggests some mechanism whereby the level in the blood is kept constant. The livers of the rats receiving oil contained, on an average twelve times as much vitamin A as those of the rats receiving no oil. The body fat of these rats was also appreciably higher in its vitamin A content. Since 13 drops, over a period of 13 weeks, were given, it seems almost certain that a great proportion of it was stored.

The results of experiment 3 (table 3 and fig. 1) show a definite accumulation of vitamin A, after increased dosage, in the liver of adult rats. Despite the fact that the animals in this experiment were killed at intervals of 28, 137, 97 and 64 days after the administration of the last drop of halibut liver oil, the curve of vitamin A concentration in the liver (fig. 1) steadily rises with doses of 4, 6, 7, 9 and 11 drops. There is no indication that vitamin A was liberated in any appreciable amount from these livers during the experiment.

It is interesting to speculate as to the reason for this striking capacity of the liver to store vitamin A. We think at once of a storage to combat infection, but our results lend no support to the theory of the anti-infective value of vitamin A, when supplied to these rats in adult life. A mild infection of the ears was found in 31 per cent of the animals on the Sherman B diet and in 32 per cent of those receiving added vitamin A (table 2). The addition of vitamin A to the diet of this latter group did nothing to prevent or cure this infection. There still remains the possibility mentioned above that these animals did not receive enough vitamin A in infancy. It is quite possible that the effectiveness of vitamin A to combat infection is determined by the age at which it is given.

Drummond, Coward and Watson ('21) observed that the colostrum of cows was considerably richer in vitamin A than the later milk. These investigators state: "It may indicate

that the child during the first few days of life requires a relatively high concentration of certain substances, amongst them vitamin A, either for its immediate requirements or to provide it with a good reserve at the outset." Similar observations were made later by Luce ('24). These early observations have never been satisfactorily explained. Our results show that the livers of the rats fed on the Sherman B diet only were not stored to capacity with vitamin A, because at 57 days and at 264 days it was possible to raise the vitamin A concentration in the liver by feeding a supplement of this factor. Furthermore, figure 1 indicates that in adult life the livers were capable of storing at least ten times the number of units obtained from the diet only. What the significance of this fuller storage is we do not know, but the fact that the liver has such a great capacity for storage suggests some physiological significance.

When the results of experiment 4 (table 4) are compared with those of rats nos. 89, 90 and 91 (table 1), a definite effect of feeding halibut liver oil to the mother is seen. The average values of vitamin A per 100 gm. liver in rats fed the diet alone was 303 units; that for rats of the same age whose mother had received halibut liver oil, 544 units. In the latter groups higher values were obtained also for blood as compared with those of the first group, and some vitamin A could be detected in the body fat. It remains to be seen, in later experiments, whether this fuller storage of the liver with vitamin A, in early life, results in better initial growth of the rats and protection against the occurrence of spontaneous infection.

In experiment 5 a wide distribution of vitamin A was found in the different tissues examined. Its concentration was surprisingly high in adrenals and lung tissue. The total amount recovered by subtracting that present in the three rats that had received no extra halibut liver oil from those that had been fed the supplement was 77 per cent of the total units fed, and of this 70 per cent was recovered from the liver. In studying the distribution of vitamin A in such a wide range

of tissues, one wonders why certain cells seem to have more of an affinity for vitamin A than others. Further experiments are being planned to elucidate this. It would seem that by our method of analysis a wider distribution of vitamin A in the body has been detected than that observed by other investigators (Moore, '30). It is certainly clear from table 5 that in every case where some vitamin A was found in the organ of the control rats, a definitely larger amount could be detected in the same organ after feeding a large supplement of vitamin A.

CONCLUSIONS

1. By a modification of the Price-Carr method for the determination of vitamin A we have studied the vitamin A content of livers, blood and body fat in young and adult rats fed on the Sherman B diet.

2. Comparing values for vitamin A obtained at 54 days and at 264 days a definite storage of the vitamin A in the liver and body fat was obtained in rats fed the Sherman B diet only.

3. The values obtained at 54 days in the liver are thought to be low, and at this age no vitamin A was found in the body fat. It is suggested that this diet may not contain enough vitamin A to supply the needs of the rat in early life.

4. An immediate storage of vitamin A in the liver and body fat was obtained by feeding 1 drop of halibut liver oil to three young rats 24 hours before death.

5. Eleven adult rats, receiving 1 drop weekly of halibut liver oil for 13 weeks, showed twelve times as much vitamin A in their livers as compared with thirteen control rats from the same litters which received no supplement to the Sherman B diet.

6. The concentration of vitamin A in the blood is no indication of the amount that may be stored in the liver.

7. No effect of vitamin A to prevent or cure the occurrence of a mild infection of the ears in adult rats was observed.

8. Increased storage of vitamin A with increased dosage of halibut liver oil was observed in the livers of adult rats. There was no evidence that this stored vitamin A was liberated from the liver during a period of 28 to 137 days after feeding the vitamin A supplement.

9. By feeding a total of 4 drops of halibut liver oil to a mother rat, 1 drop during pregnancy and 3 drops during lactation, the concentration of vitamin A in the livers of her seven young rats at 54 days old was considerably raised, as compared with that found in three young rats of the same age whose mother had been fed no supplement.

10. Analysis by our method of a number of tissues of the rat for vitamin A showed a wide distribution of this vitamin. We obtained, in terms of units of vitamin A, a recovery of 77 per cent of a large dose of halibut liver oil fed to two rats, as compared with that present in the tissues of three control animals that had been fed no supplement.

We should, in conclusion, like to express our thanks to Dr. Carl Nielsen of the Abbott Laboratories for his kindness in supplying us with the halibut liver oil used in these experiments; and to Mrs. Ruth Teasdale Hanner for technical assistance in the routine care of the animals.

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ENDOCRINE STUDIES

XLIII. THE GASEOUS METABOLISM OF SOME DWARFS AND GIANTS

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FIVE FIGURES

(Received for publication September 2, 1933)

During the visit of a circus troupe to Boston, through the courtesy of the several members of the group, the writer had the unusual opportunity of measuring the respiratory metabolism of a group of dwarfs of adult years. In addition, a number of the other members of the company, an acromegalic giant, a 'fat lady,' two alleged albino negroes, and a South African bushman, all contributed a number of measurements. In the case of the giant, J.E., a fairly complete objective study was made, the results of which have been reported elsewhere (Rowe and Mortimer, '34). Extensive radiographic studies were made of all of the above and the results are shortly to be reported by one of my colleagues. The present paper will be limited to the results of the respiratory metabolism study in which five dwarfs, the giant and the 'fat lady' took part. All of these represented different types of abnormal pituitary function; for purposes of contrast and extension, I have included the data from three other patients who in earlier years were completely studied in connection with other investigations. One of these was a massively obese boy, the other two, women with marked retardation of their physical development. For purposes of orientation, the significant physical measurements of the several members of the group are collected in tabular form.

TABLE 1
Physical measurements

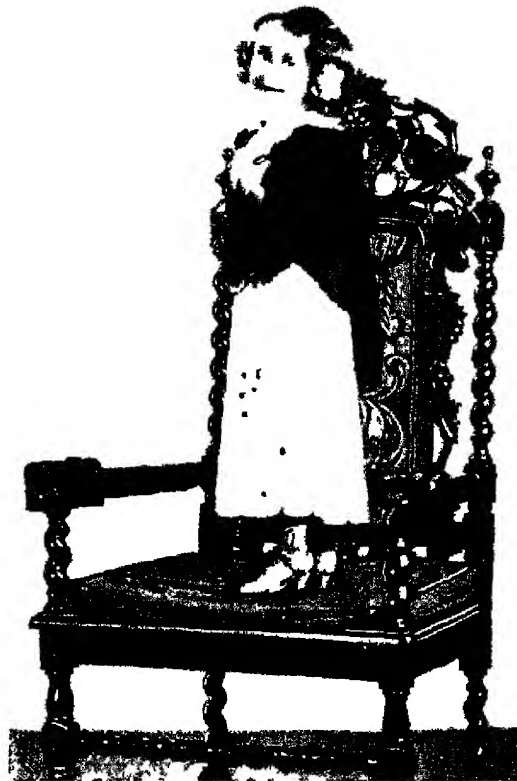
NO	NAME	SEX	AGE (YRS)	HEIGHT (CM)	WEIGHT (KG)	AREA (SQ M)	SITTING HEIGHT INDEX
1	L.G	F	22	95.0	16.3	0.639	0.566
2	T.E	F	18	107.3	19.5	0.753	0.537
3	G.E.	F	34	113.6	21.8	0.823	0.511
4	D.E.	F	26	125.8	28.4	0.992	0.523
5	M.H. ¹	F	21	140.0	43.9	1.289	0.530
6	A.H. ¹	F	17	141.7	33.4	1.158	0.550
7	H.E	M	31	107.7	21.1	0.781	0.544
8	B.G. ¹	M	14	147.2	189.5	2.489	0.603
9	R.P	F	24	160.3	182.1	2.604	0.563
10	J.E	M	26	228.6	163.3	3.125	0.483

¹ The interpolated cases

The first four in the series as well as no. 7 are professional dwarfs, no. 9 was the fat lady and 10, the giant. The several figures speak for themselves; only the sitting height indices (i.e., the total divided by the sitting height) call for any comment. My own averages for groups of several thousand men and women of relatively normal configuration are 0.524 for the male and 0.533 for the female. These values are closely in accord with those of Pfitzner ('22) who reports the same value for the male and 0.528 for the female, together with yet other values from similar sources. Recognizing that these are average values only, defining a median in the zone of the normal, a tolerance of ± 0.020 may be conceded as permissible limits of variation within the convention. Extreme values from my own series (unpublished data) are respectively 0.648 in the male and 0.656 in the female, these being achondroplastic dwarfs while the antitheses are presented in so-called 'eunuchoid' types of pituitary disease with 0.476 and 0.483, respectively. Reverting to the present series, nos. 1, the smallest dwarf, and 9, the fat lady, show high indices, while nos. 2, 3, 4 and 7, all members of one family, fall within the conventional normal zone. The fat boy, no. 8, with the very high index, is an artefact as his huge weight operating since birth had produced marked deformity of the lower limbs. For this reason his total height was not repre-

sentative as it made no allowance for the bowing of the legs. The proportions of the giant are characteristic of this condition in which the longitudinal growth is most marked in the long bones.

A brief comment on each member of the series may aid in further orientation and assist in the interpretation of the values measured for the individual energy exchange.



1

No. 1, L.G., is the youngest of three daughters, of whom the oldest, A., age 24, is also a dwarf but has the further handicap of a retarded mentality. The second girl, Lo., is 23 years old, of normal size, and entirely normal in all other respects. The subject of this study, L, was normal at birth but failed to grow so that "at the age of 6 years she could walk under a table without bending." Her teeth were very late in appearing, and she had never matured. The oldest sister, A., has menstruated a few times. The parents are people of normal size and the family history is in no sense remarkable.

Nos. 2, 3, 4 and 7, as already noted, are all members of the same family. In addition to these four, their parents had four more daughters and another son. The average height of the girls in this latter group is 5 feet 6 inches, while the brother is 5 feet 8 inches tall. Both parents are alive, seemingly entirely normal people as are the siblings noted above. The maternal grandfather is reported as having been 6 feet 2 inches tall and the mother, herself of average stature, has three brothers all over 6 feet in height



4 3 2 7

None of the three dwarf girls has matured but one of them, D., the largest of the group, complains of feeling unwell at monthly intervals and remains in retirement for 2 or 3 days. The brother states positively that there is no flow and that the whole performance is a tribute to convention on the part of the sister. The brother, H., states that he has matured and is sexually potent. Reports from other sources render this statement highly improbable.

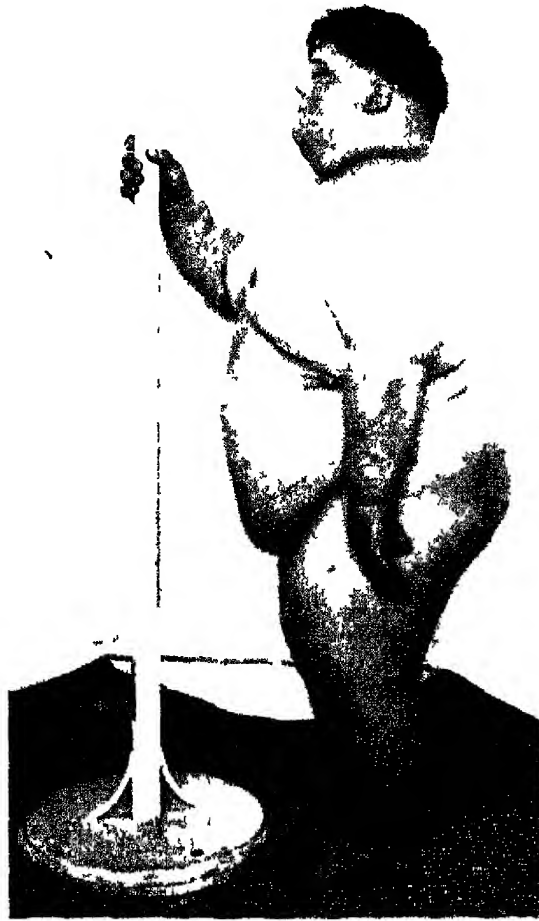
They all show an excellent level of mentality, the more so as they have been professionally engaged since childhood,

following a necessarily nomadic existence which has offered but limited educational opportunity. All of the group were normal at birth—the youngest, T, is said to have weighed about 7 pounds—but all failed to grow significantly after the age of 6 or 7. An exception possibly should be made in the case of G. who is reported to have gained nearly an inch during the last year.



Cases 5 and 6, as previously noted, were not in the original group but were patients seen in years gone by in the diagnostic service of the institution. Both had failed to develop physically and neither had matured. The younger, A.H., has had several later contacts; she has failed to grow significantly and has never matured. Pelvic examination has shown a very marked hypoplasia. These women have been added to the study for several reasons, the first of which being that in size they bridge the gap between the midgets and the normal.

Further, neither of them has matured, thus establishing a parity for comparison in this important respect. Finally, the younger, A.H., is also a case of pituitary arrest, as are the dwarfs in the professional series. The other, M. H, was a premature child and presents a constitutional inferiority in no way associated with primary ductless glandular involvement. There is a definite probability of a congenital specific



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factor which could not be verified as lumbar puncture was denied. Comparison of the respiratory metabolism of this individual with that of the others, all endocrine cases but superficially at least so like her, offers a control for the more precise interpretation of the results obtained.

No 9, the 'fat lady,' had a family history of obesity, the father, a man of 6 feet, weighing 235 pounds, while the mother, only $\frac{1}{2}$ inch less than her husband, weighs 400. R. is an only

child. She reports herself as weighing 16 pounds at birth and she has continued to gain ever since. She matured at 11, and the menstrual history as reported is entirely normal in all details. She is a graduate both of high school and of a business college.

No. 8, the fat boy, B G., was first studied at the age of 10 at which time he weighed nearly 400 pounds. Pituitary medi-



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cation coupled with reasonable dietary restraint (hospitalized) reduced him to a level at discharge of 142.3 kilo. Discontinuance of his opotherapy and his diet soon brought him back to his original level and at the time of last contact, here reported, he was just short of 190 kilo. He has since died and the newspaper report of his death gave his weight as 250 kilograms—a value that seems highly probable.

The final member of the original group, no. 10, is the giant, J.E. Beyond his great height, largely achieved between his

eighth and sixteenth years, he presents a relatively normal history. A number of his forebears were very tall people but none of them, so far as he knows, even approximated his own towering altitude. He too is a high school graduate and in his subsequent professional career has availed himself of the educational opportunities afforded by extensive travel.

It is a point to be emphasized that all of this group with the exception of the fat boy, no. 8, were of an excellent intellectual capacity which enabled them to cooperate fully in the performance of the various tests. In the case of B.G., we had a series of extended contacts with him over a period of 4 years and educated him as a subject for metabolism measurements, in which capacity he finally became very proficient.

Turning now to the actual oxygen consumption of the several members of the group, the data are collected in table 2.

TABLE 2
Observed metabolic rate

NO	NAME	OXYGEN PER MINUTE	CALORIES		
			Per hour	Per square meter per hour	Per 24 hours
1	L G.	79 0	22 9	35 8	549
2	T E	76 6	22 2	29 4	532
3	G E.	82 8	24 0	29 1	575
4	D E	109 4	31 7	31 9	760
5	M H.	187 4	54 3	42 1	1302
6	A H.	131 5	38 1	32 9	914
7	H E	90 0	26 0	33 4	625
8	B G	360 1	104 3	41 9	2520
9	R P	356 4	103 2	39 6	2476
10	J E	419 7	123 7	38 5	2969

For the first seven members of the series, and for no. 9, the closed circuit method was followed, using the original type of Benedict-Collins' ('20) apparatus with the internal motor, the form which in my experience yields the most satisfactory results. During his periods of study, both the closed and open circuit methods were used with B.G., the results obtained by both methods being mutually concordant.

Due to an earlier unpleasant and unsuccessful experience with a small closed circuit apparatus, J.E. expressed a preference for the open circuit approach and a series of measurements were made with the Tissot spirometers, analyzing the respired gases with the Carpenter modification of the Haldane apparatus. This approach permitted the measurement of the respiratory quotient, in the present instance valuable chiefly as an index of the basality of the patient's state. As noted above, he had had an unfortunate experience some years earlier and the memory of this, operating on a general condition of nervous instability, engendered some disturbance during the first measurement. This gave an oxygen consumption of 449.8 cc. per minute with a respiratory quotient of 0.97. During the second test, he was much more at ease and the oxygen consumption fell to 439.2 with an R.Q. of 0.89. The third essay lowered the oxygen still further to 419.7 cc. but with unchanged quotient. The figure given for the 24-hour heat production in the table above is based upon the calorific equivalent for oxygen at the R.Q. of 0.89 or 4.912 Cal. In my opinion, although the repetition of the test had done much to sedate the patient, he was still slightly nervous and his higher carbon dioxide output was an expression of a respiratory effort still in excess of his needs. Calculating heat production on the basis of the conventional R.Q. of 0.82, the heat exchange becomes 2916. While this is probably nearer his basal requirement, I do not feel that any of the measurements were truly basal and am certain that his real requirement is inferior to the lowest here recorded. It would be inapposite to interpolate any extended discussion of the influence of nervous tension on the apparent oxygen requirement but any one who has had experience with the vicissitudes of clinical calorimetry will recognize the magnitude that such errors may assume. Recognizing, then, that the value reported for J.E. is probably superior to his basal requirement, I have preferred to use the data which the actual measurements warrant.

Taking the first seven members of the series as presenting various stadia of substantially the same physical status, i.e., arrested development, and using the standing height and weight as convenient and significant correlation factors, the energy requirement can be calculated in terms of the units of height and weight.

TABLE 2 a

NO	NAME	TOTAL CALORIES DIVIDED BY	
		Height (cm.)	Weight (kg)
1	L.G.	5.78	33.7
2	T.E.	4.96	27.3
7	H.E.	5.80	29.6
3	G.E.	5.06	26.4
4	D.E.	6.04	26.8
6	A.H.	6.45	27.4
5	M.H.	9.30	29.7

While the empirical relationship with height fails to show any marked constancy—a fact which could have been predicted from the probable individual variations in the level of pituitary function—they are all of the same order. This is emphasized by the complete lack of agreement of the coefficient for M.H., the only member of the group with a normally functioning gland.

On the other hand, the comparison on the basis of weight shows a much more definite constancy; here no. 5 falls within the group, while L.G., a clear-cut pituitary case, furnishes the sole significant departure from the mode. As a matter of fact, both of these approaches are artefactual as they, of necessity, fail to consider varying levels of the gland function and other matters pertinent to the influences operating to produce the observed oxygen requirement.

While absolute values are significant as representing the specific performance of the individual, they assume their chief meaning when used as the basis for comparison with those quantities which have been calculated from the prediction formulas of various authorities as indicating the probable

levels of normal performance. In order that these criteria of the normal may be justly representative of the several schools of thought, I have selected those offered by Harris and Benedict ('19), by Aub and DuBois ('17) using the surface area formula of the DuBois and DuBois ('16), the Boothby and Sandiford ('29) modification of the original Aub-DuBois standards, and finally, those offered by Dreyer ('20).

In addition, as the physical characteristics of the dwarfs partake of those weights and areas usually associated with childhood, I have made rough comparisons from the curves for these correlations as prepared by Benedict and Talbot ('21). For purposes of a convenient unity, all standards have been computed to yield normal energy requirement in calories for a period of 24 hours. While this is essentially a convention and does not actually reflect the conditions of living for that period, custom has sanctioned it as acceptable. Further, it can be translated into any other method of expression by simple arithmetical adjustment.

TABLE 8
Normal prediction

NO	NAME	H-B.	A-DUB.		MOD. A-DUB ¹		D.
			Unit	Prediction	Unit	Prediction	
1	L.G.	884	37.0	567	36.9	566	769
2	T.E.	956	38 0	687	37.3	674	844
3	G.E.	914	36.5	721	36.2	715	820
4	D.E.	1038	37 0	880	36.6	871	970
5	M.H.	1236	37.0	1145	36.9	1142	1241
6	A.H.	1157	40 0	1112	37 4	1039	1114
7	H.E.	686	39.5	740	39 8	746	905
8	B.G.	3313	46.0	2748	46 2	2760	3017
9	R.P.	2410	37.0	2402	36.9	2396	2330
10	J.E.	3279	39.5	3048	40 3	3110	3051

¹ As changed by Boothby and Sandiford ('29).

As was to be expected, the prediction values show a wide variation. In the main, the Harris-Benedict normals spell one extreme and the two sets based upon surface area the

other. A sole exception to this is in the case of the male dwarf, H.E., whose H.-B. prediction is significantly the lowest of the four. That the two surface area values should show excellent agreement is inevitable, as the modification consists solely in changing slightly the original values for the heat production per square meter per hour. Dreyer's predictions fall between the two extremes—a fact again to be anticipated from the sources of his data and its method of handling. A sole significant exception is the case of the male dwarf, H.E., where the H.-B. is the lowest and the Dreyer significantly the highest prediction value.

As was noted above, rough comparisons have been made where possible with the Benedict-Talbot values for weight and area. The predictions have been taken from the curves and lack the precision of the other calculated values.

TABLE 3 a
Benedict-Talbot predictions

NO.	NAME	WEIGHT	SURFACE
1	L.G.	705	660
2	T.E.	795	755
3	G.E.	850	825
4	D.E.	1005	960
5	M.H.	.. .	1265
6	A.H.	1125	1125
7	H.E.	900	845

The agreements here are good, the common origin of the two methods of approach rendering this certain.

Turning finally to the comparisons of the rates actually observed with those predicted by the several formulae, we have an approach at least to the function levels of the individuals composing this group.

Dealing first with the dwarfs, there can be no question that they all show a downward tendency to the energy requirement which assumes very concrete proportions in several instances. The smallest of the group, L.G., shows a lack of agreement between the two surface area formulae which are normal,

TABLE 4
Deviations from prediction (per cent)

NO	NAME	H-B	D	B T		A-DUB.	A-DUB. MOD.
				Weight	Surface		
1	L.G.	—38	—29	—22	—17	—3	—3
2	T.E.	—44	—37	—33	—30	—23	—21
3	G.E.	—37	—30	—32	—30	—20	—20
4	D.E.	—27	—22	—23	—21	—14	—13
5	M.H.	+5	+5	+3	+14	+14
6	A.H.	—21	—18	—19	—19	—18	—12
7	H.E.	—9	—31	—31	—26	—16	—16
8	B.G.	—24	—17	—9	—9
9	R.P.	+2	+6	+3	+3
10	J.E.	—11	—4	—4	—6

and all of the other predictions which are significantly depressed. The E. family are all consistently low, the Harris-Benedict comparison of the brother being the only one that falls inside the conventional normal boundaries. It is perhaps meaningful that the degree of the depression in the three girls parallels that of the arrest in their development. D., the middle sister, is the tallest and shows the least depression although even she is at a frank hypofunctional level. The interpolated pituitary case, no. 6, A.H., approaches the levels shown by D. The other interpolated case of arrest, M.H., with no evidence of endocrine involvement is above prediction in every instance, and approaches the upper normal boundary in the case of the two surface area predictions. The biometric differences between this patient and A.H. are slight, and again between the latter and the largest of the E. family, G., the gap is not unbridgeable. While this paper was in preparation, a patient was referred to the clinic in the person of a woman of 29, whose height is 135.3 cm. while her weight is 45.0 kilo. Her observed energy requirement was 964 cal., the Harris-Benedict prediction 1200 cal., and the modified Aub-DuBois 1107 cal. These correspond respectively to —20 per cent and —13 per cent below prediction. It has been the practice of this institution to compare observed rates with both the Harris-Benedict and Aub-DuBois¹ standards, taking

¹ More recently the Boothby-Sandiford modification has been substituted

the mean of the reported value. On this basis the patient shows a rate of — 17 per cent strictly comparable to that of A.H. Thorough diagnostic study of this latter patient shows a long-standing pituitary dysfunction to be the etiological background of her underdevelopment. She has matured, however, and has had one miscarriage together with one child who died 5 minutes after delivery. An acquired venereal factor is undoubtedly responsible for these mishaps. The case is introduced here as she bridges still further the gap between the dwarfs and normal sized adult individuals and offers further warrant for confidence in applying conventional normal standards to this group of pituitary developmental arrests.

Turning to the three remaining cases, we have the two obese individuals, both of whom have a pituitary background. In the case of the boy, there is a long-continued record of study covering intermittently a period of over 4 years. His energy requirement was always below prediction, and in some of the reports during this interval fell to levels very appreciably below the one recorded in this study. The girl, R.P., is in a present transition state and while her true level is certainly inferior to that recorded, she is probably for the moment not far below prediction. The agreement between the several comparisons is interesting.

The final case, the acromegalic giant, gave values which were certainly too high. This point has already been touched upon in the body of the paper. He, too, represents a transition phase of glandular activity in which an initial overproduction of at least the growth hormone is now spontaneously undergoing functional involution to a terminal state of hypofunction.

While all of these measurements are open to the criticism that so warrantably applies to such records with all unconditioned subjects, they yet are maxima and the establishment of a truer physical and mental relaxation would do no more than lower still further readings already low.

In an early paper, Aub and DuBois ('17) had reported the results of studies of a group of individuals of unusual configuration which included five dwarfs. One of these (P.W.) was rachitic, two (R.deP. and S.G.) were achondroplastic, and two others (I.E. and G.F.) were endocrine cases. In addition, they studied the case, J.P., a 17-year-old boy reported by McCrudden and Lusk ('12-'13) as a case of Herter's ('08) 'intestinal infantilism.' The data from one of the achondroplasias (S.G.) do not lend themselves to further scrutiny as the patient was not well and the test abruptly terminated. The data from other five subjects have been calculated in terms of the present approach and the significant findings are collected in the next table.

TABLE 4 a
Analysis of Aub-DuBois data

NAME	AGE	HEIGHT	OBSERVED (INDIRECT)	H-B	D	A-DUB. (MOD)
P.W.	38	123 8	1180	941 + 25%	1172 + 1%	1129 + 4%
R.deP.	35	134.7	1256	1066 + 18%	1240 + 1%	1167 + 8%
I.E.	32	134.0	828	1034 - 20%	1200 - 31%	1118 - 26%
G.F.	48	148 9	1159	1216 - 5%	1354 - 14%	1370 - 15%
J.P.	17	113 3	746	811 - 8%	986 - 24%	853 - 13%

The same inconsistencies in prediction values are found here as in the other series. At the same time, the general trend of the indications is unmistakable. P.W. and R.deP., cases without ductless glandular involvement, show values above prediction and with the exception of the H.-B. values, well within normal limits. I.E. was an endocrine case (hypo-functional) and shows a characteristic depression of the respiratory metabolism. G.F., also an endocrine case, gives less well-marked evidences of departure from the normal, so

far at least as the respiratory exchange is concerned. He is, however, 48 years old, and his height non-significantly below 5 feet—in other words, the energy requirement is consistent with his pituitary status. The last case, J.P., without glandular involvement, but in a state of marked malnutrition, shows the depression of oxygen use which reflects the nutritive status. This group of cases, studied independently and by both direct and indirect calorimetry, yield values which are wholly consistent, in relation to the physical status, with those of the present series.

As was noted in the beginning, certain other aspects of the studies on this interesting group will be reported elsewhere. The present paper is confined to the report of the respiratory metabolism.

One other group of observations needs be recorded. In all clinical metabolism measurements there are a number of significant physical records that constitute an essential complement to the measurement of the oxygen consumption. From these records, taken at intervals throughout the time of contact with the subject, some estimate can be made of the degree of relaxation at the time of testing, and hence of the authority of the energy consumption report.

TABLE 5
Complementary physical data

NO.	NAME	BLOOD PRESSURE		PULSE	RESPIRATION	TEMPERATURE
		Systolic	Diastolic			
1	L.G.	94	68	80	10	98.0
2	T.E.	88	52	76	12	98.4
3	G.E.	106	80	64	14	98.0
4	D.E.	86	50	64	15	98.0
5	M.H.	102	58	76	16	99.2
6	A.H.	102	70	84	14	98.0
7	H.E.	98	72	79	16	98.6
8	B.G.	164 ¹	90 ¹	64	16	97.2
9	R.P.	160	90	62	7	97.8
10	J.E.	146	90	78	10	98.6

¹ Known cardiorenal condition.

In the main, these values warrant confidence in the relative accuracy of the energy metabolism reports. Case 5, M.H., has a slightly high temperature but it will be remembered that her comparisons were all slightly above prediction. Correction to a conventional 98° will still leave them all within the normal zone. The fat boy was a cardiorenal case and seemingly the fat girl has an early hypertension. The somewhat high diastolic pressure of the giant is in harmony with the hypertensive influence of pituitary overactivity. Case no. 6, A.H., shows a pulse somewhat above her usual level. Broadly speaking, the pituitary dwarfs show hypotension and are otherwise fairly normal. This is in complete accord with the usual clinical experience.

To conclude, we have here a record of the respiratory metabolism of a group of individuals all of whom depart significantly from conventional physical standards. The basal rates, in general, reflect the physical status that has engendered their abnormality. Further studies, which it is hoped to make, will probably show somewhat lower values, but it is questionable if the further depression will assume magnitudes significantly different in comparison with those here recorded.

I wish to express my deep appreciation of the courtesy and friendly cooperation on the part of the subjects which made these studies possible, recognizing that it entailed a sacrifice of leisure and of personal convenience.

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THE TOXAEMIAS OF PREGNANCY

III. THE RESPIRATORY METABOLISM

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TWO FIGURES

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In a series of earlier communications (Rowe, '32; Rowe et al., '33), the senior author and his associates have discussed certain results derived from the study of fifty-two pregnant women exhibiting some one of the toxaemias which may complicate the physiological condition.

The present report is concerned with the respiratory metabolism together with certain other observations germane to its discussion. The general method of approach, composition of the group, and similar material, have already been presented in the earlier papers and need not be detailed here. It may be noted, however, that each of the group was studied at some time ante-partum and again within 2 weeks after terminating the pregnancy. All of the women were toxic, one died 9 days after delivery, and two more died during the initial study, the records being so incomplete that they have been deleted from the series. As the relationship of the time of initial study to that of delivery is pertinent to certain aspects of the discussion, the data are briefly presented.

TABLE 1

<i>Group</i>	<i>Number</i>
A. Delivered at time of study,	33
B. Delivered within 1 month later,	6
C. Delivered from 6 weeks to 6 months later. Average 3.5 months,	11
	<hr/> 50

Two-thirds of the series were delivered spontaneously or by induction at the time of first hospital entry; six more terminated the pregnancy within a month of the initial study. With the remainder, one-fifth of the group, treatment permitted a continuance of gestation. Of the eleven children in this group, two were delivered at term spontaneously, one of which was a stillbirth, while the other survived only 16 hours. Labor was induced in two others, delivering a dead foetus at 6.5 months in one, and an 8 months' child which survived 6 days in the other. The remaining seven children survived. The complete record of the outcome of pregnancy is given in the next table.

TABLE 2
Outcome of pregnancy. Toxaemia series

DATUM	DELIVERY		
	Spontaneous	Induced	Total
Premature, survived	4	5	9
Premature, dead	2	7	9
Premature, died later	2	5	7
Full term, survived	13	8	21
Full term, dead	2	1	3
Full term, died later	1	0	1
	24	26	

Twelve pregnancies yielded dead children ranging from a therapeutic abortion of a 2½ months foetus to the child still-born at term. Eight more were living at the time of delivery but died after intervals ranging from 5 hours to 28 days. A more complete analysis of these two groups will be given in the body of the paper in connection with the respiratory exchange.

Prior to undertaking the investigation of toxic pregnancy, a series of standardizing studies on normal pregnant women was conducted by the senior author and his associates. The results have been summarized (Rowe, '31) and later reference will be made to these studies of the normal as they serve as the control material for the present investigation. During

pregnancy, a variety of mechanisms are engendered which are associated with the underlying physiological condition and which terminate with delivery. The growth of the foetal tissues, the storage of protein by the maternal organism in excess of foetal needs, and a variety of other processes all influence the energetic demand of the maternal organism which during the period of gestation comprehends them all. That the added foetal metabolism alone is not competent to account for all the changes recorded in the measurement of the energy requirement of the mother, has recently been shown by one of us (Rowe and Boyd, '32). The growing foetus, however, does make a significant and highly important contribution to the factors which can be measured only in summation. Weights at delivery, as potential rough measures of the foetal contribution, assume a very real importance for present considerations. Average values for the several groups have been collected; the average weight of the seventy-seven children in the normal series is included.

TABLE 3
Weight relations, child

DATUM	NUMBER	WEIGHT (AVERAGE)
		<i>kg.</i>
Premature child, dying after delivery	7	2.15
Same, surviving	9	2.62
Full term child, surviving	21	3.38
Same, normal series	77	3.27

The stillborn children, twelve in number, have been omitted as the records are incomplete and the scatter of weight and period of gestation is scarcely significant in that portion where report was made. The record is omitted from the table also of one child spontaneously delivered at term which survived 16 hours. This was the largest baby in the series, weighing 4.34 kg.

The non-surviving premature children present one definite handicap in an average weight nearly one-fifth less than that

of the surviving series. The full term children, on the other hand, show a slightly greater average weight than the babies of the normal series, and this in spite of the subversive influence that the mother's toxic state might be presumed to exercise on foetal growth. While the exact degree of prematurity must be a matter of estimation and so lacks any real precision, the weights as recorded are equal or slightly superior to those reported by Scammon and Kline ('30). A quantitative comparison is impossible but qualitatively at least the surviving children have shown a normal development in spite of the mother's condition; one explanation of the lighter non-surviving group could patently lie in a greater prematurity than their surviving fellows.

While the foetal weight cannot be recorded with precision, that of the mother is not subject to this disability if certain reasonable precautions are taken to render the records of a series comparable with each other. Further, the maternal weight summates all of the tissue changes and accretions deriving from the fact of the pregnancy. The data from all of those with complete records are collected in table 4. The complementary values from the normal group are added for purposes of comparison.

TABLE 4
Maternal weight relations

DATUM	NUMBER ¹	WEIGHT (KILOGRAMS)		LOSS
		Ante-partum	Post-partum	
Delivered:				
At term	9	78.9	66.6	12.3 (16%)
1 month premature	8	72.0	62.2	9.8 (14%)
2 months premature	12	68.4	59.6	8.8 (13%)
Over 2 months premature	10	59.5	58.9	0.6 (1%)
Normal:				
At term	77	66.3	57.4	8.9 (13%)
1 month premature ²	77	64.4	57.4	7.0 (11%)
2 months premature ²	77	62.5	57.4	5.1 (8%)

¹ In eleven of the toxic patients, only single weights could be secured, which unfits them for this comparison.

² Calculated from observed weekly coefficient of 0.48 kg.

In the toxic group, one finds those increments of weight with the progress of gestation which are to be anticipated. They are somewhat greater in the toxic than in the normal group as gauged by the average weights 2 weeks post-partum. The warrant for computation by a linear formula for the latter group has already been established in the paper previously cited (Rowe and Boyd, '32). While absolute values offer basic data, relative changes at times disclose an underlying trend with a greater clarity.

Some years ago, Dreyer ('21) computed a series of relationships from observations on a group of normal individuals which are of real service, within certain limits, in the prediction of certain normals. Dreyer calculates weight from sitting height ('trunk length') and chest girth, the former uniformly valid with reasonably normal sitting height indices, the latter applicable only, as one of us previously has pointed out (Rowe, '25), to people of relatively normal configuration. The normal sitting height index (sitting height divided by total height) shows an average of 0.533 for adult women in this part of the world. Reasonable deviation within the normal could lie between 0.52 and 0.54, and with these indices Dreyer's predictions are to be regarded as reliable, as are the measurements from which they derive. In the present group of toxic mothers, the indices are grouped as in the next table.

TABLE 5

Sitting height indices

Index	0.50	0.51	0.52	0.53	0.54	0.55
Number	3	5	12	19	9	2

In other words, 80 per cent of the group falls within the rather limited arbitrary normal boundaries and the remainder, while their indications are somewhat erroneous, cannot influence the averages of the entire series to a disturbing degree. Translating the absolute values of table 4 into the relative magnitudes based upon Dreyer's sitting height ('trunk') predictions, the results appear in table 6.

TABLE 6

Deviation of maternal weight from predicted normal (Dreyer)

DATUM	NUMBER	WEIGHT DEVIATION (PER CENT)		
		Ante-partum	Post-partum	Loss
Delivered:				<i>Per cent</i>
At term	9	+ 38	+ 16	22
1 month premature	8	+ 34	+ 16	18
2 months premature	12	+ 35	+ 17	18
Normal series	77	+ 15	+ 1	14

The first and most significant fact that this approach discloses is that the members of the toxic group are definitely overweight. Not only are the departures ante-partum of an order that indicates a real obesity, but the deviations after delivery, in spite of the high weight losses as given in table 4, indicate a group of individuals who are still much above the normal. The average post-partum excess is actually greater than the recorded deviation of the normal group just before delivery. Further, it contrasts strikingly with the practical coincidence of observed and predicted values in the normal series after the pregnancy has terminated. The toxic cases were obese to begin with, they added a disproportionate amount of weight—only attributable in part to foetal growth—during gestation and they remain significantly overweight after delivery. The post-partum comparisons are susceptible to a further detailed analysis and these data are collected in the next table (table 7).

TABLE 7

Relative maternal obesity post-partum

DATUM	NUMBER	AMOUNT (AVERAGE)
		<i>per cent</i>
Below prediction	12	— 10
From prediction to + 15 per cent	12	+ 10
Over 15 per cent above prediction	18	+ 32

Only twelve of the forty-two patients with complete post-partum records are below prediction, and that only with the

modest average of — 10 per cent. On the other hand, 43 per cent are not only above the liberal allowance of + 15 per cent, but their average deviation is + 32 per cent—a very tangible degree of overweight.

Now it is to be remembered that all of these women were toxic, giving a definite number of evidences of markedly disturbed function levels. Gastrointestinal disturbances were common—an agency that should lower rather than augment weight. Naturally, edema was a common finding, and this might be conceived to account for a part at least of the overweight, and, moreover, a part that could prove to be a disturbing source of error in the proper prediction of the energy metabolism. But the overweight persists after the uterus has been emptied and the edematous accumulation in largest part cleared up. Naturally, inert water trapped in the tissues will influence subversively the accuracy of any prediction in which weight plays a part. It is, however, only one factor and thus does not appear in the prediction value as more than a fraction of its absolute amount. To illustrate, 20 kg. of water from edema, raising the weight from 58 to 78 kg., would change the weight component of the energy prediction by the Harris-Benedict ('19) formula from 1210 to 1401—an error lessened when the age-height increment is added to make the total. For this group it would be of the order of 14 per cent.

A similar approach, using the DuBois ('17) surface formula, would increase the area of a 160 cm. woman (the average for this series) from 1.60 to 1.81 square meters, or about 13 per cent. Edema, then, while a factor, cannot enter into the picture in a magnitude which assumes disturbing proportions. In the post-partum period, it seems reasonable to say that its potential effect is wholly negligible.

In addition to his weight predictions, Dreyer (l.c.) has also calculated standards of normal lung volume. Here again his limitation to individuals of relatively normal configuration makes his standards based upon weight and chest circumference worthless for those who do not comply with this

criterion. The correlation with sitting height, within the limitations already noted, gives values of greater applicability.

In the paper previously noted (Rowe, '31), one of us has described the increase in lung volume exhibited by the normal woman as her pregnancy progresses. Taking a representative linear translation of this curve as a standard, we have

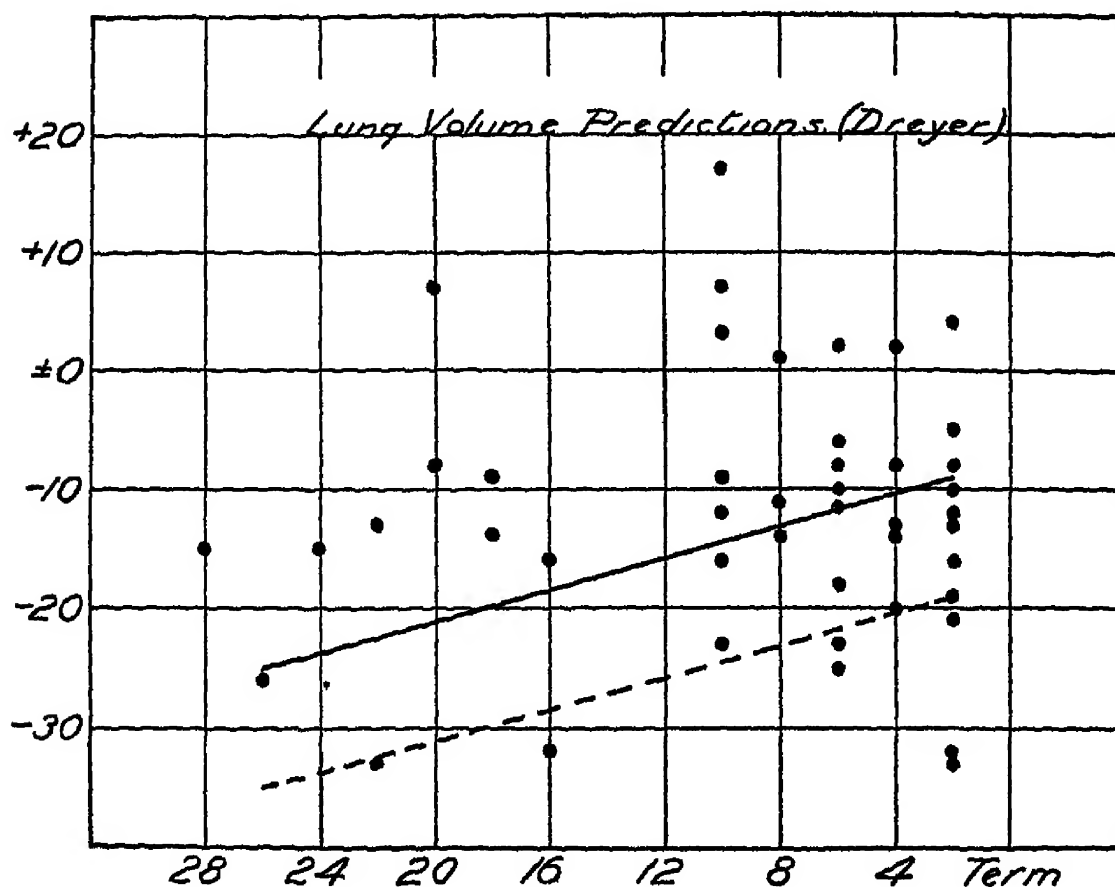


Chart 1

made a scatter diagram of the reports of lung volume deviation derived from this present toxic series (chart 1).

The heavy black line is the characteristic taken from the data of the normal series; the dotted line, a boundary 10 per cent below this standard which may be conceded to indicate a reasonable lower limit for normal performance. It is evident that but a small number (Rowe, '31) of the entire series fall below this lower boundary, and further, that twenty-five of the forty-five points are superior to standard curve. In other

words, there is no reliable evidence here to indicate a lessening of lung volume as a result of the toxic state, and during the earlier months of gestation, at least, the values for the toxic are superior to the normal average. Even at 9 months, in this the most heavily downward weighted series of observations, the normal average is —9 per cent, and that of the toxic group —15 per cent. The average deviation of the toxic group, usually about 12 days post-partum, is the nearly normal figure of —12 per cent.

During the standardizing studies on the normal group, basal rates were measured at intervals of 2 weeks from the time of initial contact to some 3 months after delivery. The composite of these measurements has defined an average curve of the deviations from prediction throughout gestation. During the last 24 weeks before delivery, these averages define a linear upward trend from a point 8 per cent below to one 5 per cent above prediction. Taking this median as the standard and defining conventional boundaries of normal deviation at 10 per cent above and below, it has been possible to construct a scatter diagram of the basal rates actually recorded with the toxic series (chart 2).

A few words of explanation may be apposite. It has been the practice of this institution in reporting basal rate measurements to compute the normal prediction by both the Harris-Benedict ('19) and Aub-DuBois ('17) equations and record the mean deviation of the two comparisons as that of report for the test. More recently, we have substituted the Boothby-Sandiford ('29) values for heat production per square meter per hour for the earlier Aub-DuBois standards. In the present instance, however, the earlier practice has been followed throughout.

To the informed group under whose scrutiny this paper will fall, it is superfluous to discuss at any length the sources of error in the measurement of the so-called basal rate with individuals who have not been conditioned to the test. Further, the patients in this group, as has already been emphasized, were sick, many of them gravely so, and this

offered a still further hindrance to obtaining authoritative results. Recognizing the subversive factors intrinsic in work of this character, every effort has been made to minimize the sources of error as far as possible. The patients were all hospitalized—a fact of great assistance in the accomplishment of this end; among other advantages, it permitted the

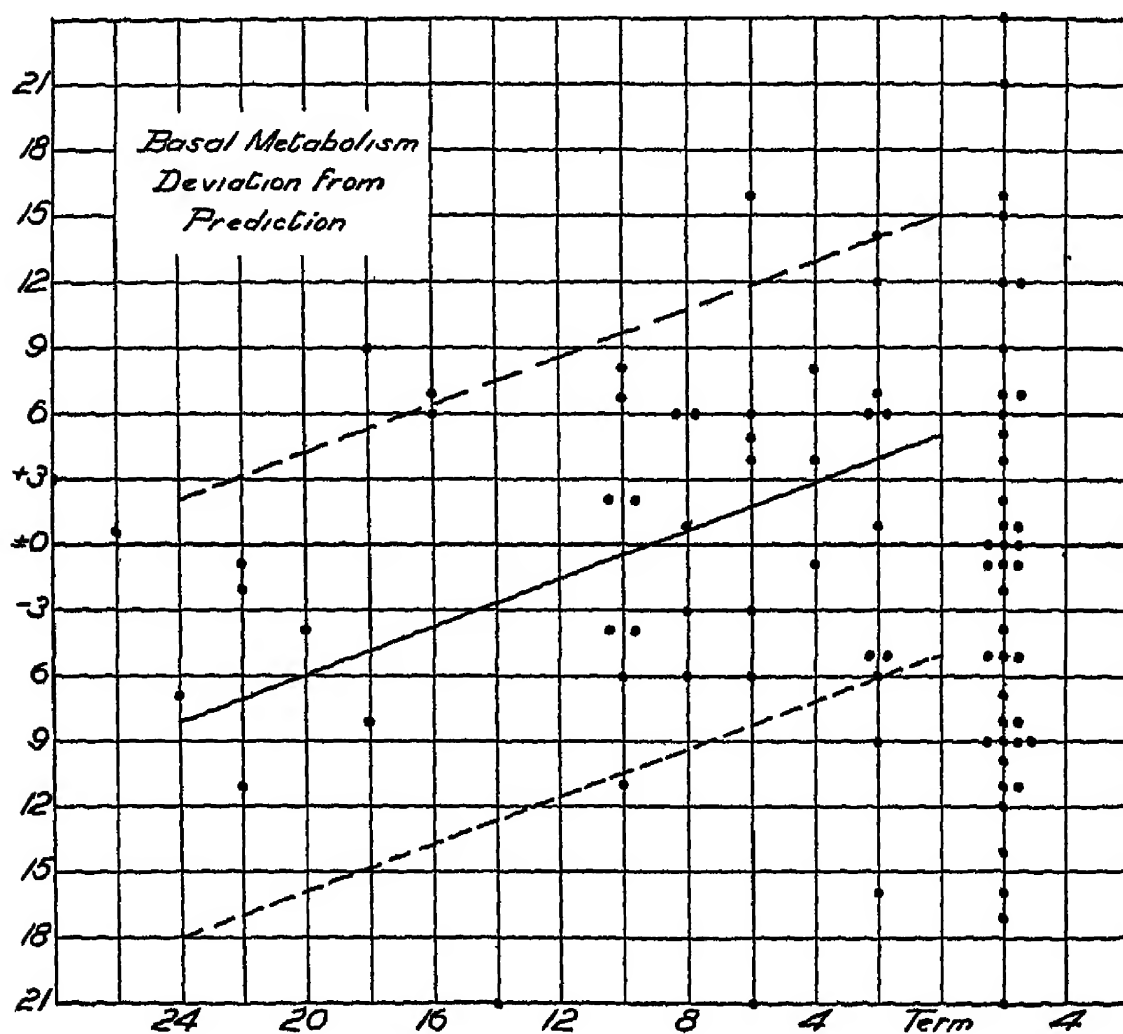


Chart 2

performance of preliminary tests which served, through education, to lessen the psychic factor of disturbance. In one or two instances, the patient was too ill to permit the performance of the test. Early in the series, we did carry out tests on a very sick and highly neurotic woman of 38, recognizing that the results were worthless as an indication of the basal energy requirement. Her best test ante-partum

was + 76 per cent and the level 12 days after the spontaneous delivery of a viable child some few weeks before term was + 27 per cent. She was in a much better state at the time of the second (post-partum) test but was obviously in a far from basal condition, restless and mentally much disturbed. She did not have a toxic thyroid. With these and the many similar observations deriving from reliable sources, the occasional reports in clinical literature of isolated measurements on obviously far from basal patients offer but meager warrant for the sweeping generalizations so freely drawn from them.

Reverting to our own data as charted in the graph, only nine of the forty-six records fall outside the conventional normal zone, four above and five below. Further, one of the low values and one of the high are but 1 per cent outside of the limiting boundaries and the single value at 28 weeks is probably well within the normal range as the curve of the normal makes a sharp upward excursion at about this point (Rowe and Boyd, '32). In other words, these data indicate that outside of the exceptional case in which some superimposed factor of influence exists, there is no reason to believe that toxaemia in pregnancy produces any characteristic influence on the respiratory metabolism. Two or three of the patients may—and probably did—have a hypofunctional endocrinopathy which would exercise its own influence quite independently of the toxic status.

The values post-partum call for a different standard of comparison. There is a definite although transitory drop in the basal rate which in the normal series gave averages of —10 per cent and —15 per cent for two individual sub-groups at intervals between 2 and 3 weeks post-partum. In the toxic group, the average interval was 2 weeks or a little less, so that the value of —10 per cent would be a fair average of normal performance. If this be assumed as the mean value, twenty-six of the forty-one records obtained fall within the conventional normal zone of ± 10 per cent. All of the others, fifteen in number, are superior to prediction and in

a number of cases—chiefly those showing the higher values above prediction—were recognized as not basal and unsatisfactory at the time of performing the test. In other words, again, neither ante- or post-partum do these results warrant any interpretation of an influence of toxæmia on the basal metabolic rate.

While the fate of the foetus is not a rigorous standard for the estimation of the severity of the toxæmia, it seems a reasonable inference that in the cases presenting a dead foetus or one not surviving after birth, the toxicity was severe. Analysis of this special group offers some measure of control over the inferences drawn from the survey of the whole group. These data are collected in the next table.

TABLE 8
Non-surviving babies

NO	MONTH SEEN	DELIVERY		DEAD SURVIVED	MATERNAL BASAL RATE	
		Type	Time		Ante-partum	Post-partum
3	3.5	Spon.	4	D	—7	—11
5	8	Ind.	8	18 days	+6	+16
10	5.5	Ind.	6	D	+7	—
13	7.5	Spon.	8	12 days	—6	+21
16	8	Ind.	8	28 days	—6	—
21	9	Spon.	9	D	+1	—
22	9	Spon.	9	D	+7	—9
29	4	Ind.	6.5	D	—2	—9
30	6.5	Ind.	7	5 hours	—	—7
31	5	Spon.	9	16 hours	—8	—
35	7	Ind.	7	D	—4	—8
36	7	Spon.	7	D	—4	+12
38	8	Ind.	8	D	—3 ¹	—
39	4.5	Spon.	8	6 days	—4	—5
40	8.5	Ind.	8.5	12 hours	—1	—
41	2.5	D. and C.	2.5	D	+3	—4
45	7	Ind.	9	D	+8	—10
46	8	Ind.	8.5	2 days	+4	+1
47	7	Ind.	7.5	D	—11	—9
50	7	Ind.	7	D	+7	—21

¹ Mother died after delivery.

The figures scarcely without exception fall within normal ranges. Nos. 5, 13 and 36 are somewhat high post-partum,

but high values lack the definitive meaning of those significantly below prediction. One, no. 50, is possibly low and is one of the cases showing the same level ante-partum. An endocrine factor here is not improbably operative. One striking case is that of no. 38, a primipara of 30 years of age. Labor was induced during the eighth month, the child was stillborn, and the mother died 9 days later. A few days before her delivery, her basal rate was the rigorously normal value of — 3 per cent. The further analysis of these selected cases certainly supports the inference drawn from the survey of the entire group.

Alveolar carbon dioxide is another element of the respiratory exchange significantly affected by pregnancy. During gestation there is a depression of CO_2 tension to levels suggesting acidosis and these continue throughout the period of gestation. The average value for the normal series was 31 mm. with recovery to 35 mm. 2 weeks after confinement. In the toxic series, the average ante-partum was 35 mm., and 2 weeks after delivery 39 mm. The relationships are the same but the toxic cases show more nearly normal values than do the normal. The probable explanation of this abnormality is not immediately forthcoming, in part at least because the initial underlying mechanism has not been satisfactorily explained. The Macallum theory of ketogenesis offers a possible lead, but discussion here is not germane to the main thesis.

There remains one other group of facts which are an essential part of every properly conducted measurement of the basal metabolic rate since they may give valuable indirect information as to the authority of the main observation. These are such physical indices as the blood pressure, pulse and respiration rates and body temperature. Before discussing these results in the present study, one further consideration must receive cognizance. The term toxæmia of pregnancy is no more than a convenient collective caption embracing a somewhat wide variety of abnormalities with only the associated physiological condition as the single, common

factor. In the majority of cases, however, evidences are not lacking of a potent hepatic or renal element, one or both, and as each of these mediates results in some measure independent of the other, due allowance must be made for each in any form of collective presentation. In the previous papers, the cases of this series have been divided into four sub-groups on the basis of hepatic and renal elements in the etiologic picture. Table 9, which follows, defines these for the subsequent discussion.

TABLE 9
Composition of toxæmia group

GROUP	I	II	III	IV
Liver	+	+	?	0
Kidney	0	+	0	+
Number	12	12	12	14
Per cent	24	24	24	28

The uniformity of distribution is fortuitous. In subgroup III, an hepatic element was possible where not actually probable, but the crucial objective evidences to resolve the matter could not be secured. Using these subgroups for greater clarity in presentation, the evidences supplementary to the basal rate record are collected in the next table.

TABLE 10
Supplementary physical measurements

DATUM	I	II	III	IV	NORMAL
Blood pressure:					
Systolic, ante-partum	146	169	148	152	108
Systolic, post-partum	141	140	149	135	112
Diastolic, ante partum	85	105	94	100	67
Diastolic, post-partum	84	90	94	83	70
Pulse, ante-partum	73	78	78	77	78
Pulse, post-partum	78	79	74	75	67
Respiration, ante-partum	14	16	16	16	17
Respiration, post-partum	15	15	15	17	17
Temperature, ante-partum	98 ⁵	98 ⁵	98 ⁴	98 ³	98 ¹
Temperature, post-partum	98 ⁵	98 ⁴	98 ⁴	98 ⁴	97 ³

All of the groups show a hypertensive trend which, in group II, with one influence supplementing the other, assumes the highest level. Recovery to normal levels after delivery is not a feature of any of them, but the two sub-series with the renal factor show a greater tendency toward conventional normality than do the others with uncomplicated and established or putative hepatic complication.

~~Pulse~~ rates, initially recording the somewhat more rapid rhythm characteristic of normal pregnancy, fail to recover in the toxic as do those of the normal series after delivery. These augmented rhythms may derive in part from the psychic influences that indubitably are responsible for some of the questionable basal rate measurements on which comment has already been passed. The respiration rates show divergences of so small a magnitude as to be non-significant. There is an upward tendency to body temperature in the toxic patients which seemingly tends to persist after the delivery of the child.

To summarize, these supplementary evidences, largely of a cardio-respiratory origin, give much more clear-cut evidences of the effect of the toxaemias than have the other observations discussed in the body of the paper.

As running comment has been passed upon each set of evidences as they were presented, further discussion is superfluous. The contents of the paper may be briefly summarized as follows:

SUMMARY

Somewhat elaborate study of a group of fifty pregnant women with some form of the toxaemias of pregnancy, observed both before and after delivery, has yielded the following tentative conclusions:

1. The toxic cases exhibit an obesity that persists after delivery.

2. The weight of the children born at term does not depart significantly from those resulting from normal pregnancy. This same generalization seemingly holds true for the pre-

mature births as compared with normal values computed from reliable sources.

3. The lung volumes of the toxic do not depart significantly from those defined as normal for the pregnant state.

4. The respiratory metabolism of the toxic patient is, generally speaking, well within the range of that determined for the course of normal pregnancy. Departures from this are few in number and are to be traced to superimposed effects not demonstrably associated with the toxic status.

5. Alveolar carbon dioxide tensions, while depressed, do not reach the low levels recorded in normal pregnancy.

6. Blood pressures, pulse rates and body temperatures all show concrete changes from the normal traceable to the toxic condition. Recovery to normal levels after delivery is delayed.

The authors express their appreciative thanks to all who have participated in these studies.

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THE EFFECT OF MINERAL OIL ADMINISTRATION UPON THE NUTRITIONAL ECONOMY OF FAT-SOLUBLE VITAMINS

II. STUDIES WITH THE VITAMIN A FACTOR OF YELLOW CORN¹

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TWO FIGURES

(Received for publication September 1, 1933)

Mineral oil ingestion by an animal under certain experimental conditions impairs the assimilation of vitamin A. In a previous communication (Jackson, '31) we reported extensive experiments demonstrating that the administration of the minimum requirement of butter fat in a mineral oil solution results in a considerable restriction of growth with other attendant symptoms of vitamin A deficiency. These studies confirm those of Dutcher, Ely and Honeywell ('27). It was shown further, however, that if the butter fat and mineral oil were given at different times, the animals gave evidence of only a slight deficiency of vitamin A. Finally, the separate administration of mineral oil apparently produced no untoward effect when the butter-fat dosage was increased by 50 per cent.

While the writer's communication was in press, there appeared a paper by Rowntree ('31) dealing with the same subject. This investigator observed that mineral oil administered with cod liver oil did not interfere with vitamin A absorption. Although no assay values are presented, the

¹ The expenses of this investigation were defrayed from the income of the Mineral Oil Fellowship, Yale University, 1929-1930.

quantity of cod liver oil employed appears to be several times the minimum requirement. When the vitamin A intake was limited to something less than this requirement, the incorporation of mineral oil with the vitamin (from 'food extracts') caused the animals to lose weight very rapidly. In general, Rowntree's results appear to be in accord with those of other investigators.

In view of the importance of the mineral oil-vitamin A question relative to the large consumption of mineral oil for the relief of constipation, we have made further studies bearing on this problem. The following report deals with the effect of mineral oil administration on the availability of vitamin A or its precursors supplied in yellow corn.

EXPERIMENTAL PART

Inasmuch as a complete description of and the arguments for the general technic employed in this study have already been presented (reference cited), only the more important items are recounted here.

Animals, diet and depletion method. Male albino rats, ranging in weight from 39 to 46 gm., were weaned at the age of 21 days and given the following vitamin A-deficient diet ad lib.

Composition of the basal diet (without added vitamin A)

	<i>Total weight per cent</i>	<i>Total calories per cent</i>
Casein, extracted	18	16
Starch, cooked	63	55
Salts, Osborne and Mendel ('19)	4	..
Hydrogenated vegetable oil ¹ .. .	15	29
	—	—
	100	100

¹ Crisco.

Vitamins B, G and D and other possible growth factors were supplied daily apart from the rest of the ration in the form of 400 mg. of dried yeast (Northwestern Yeast Company) irradiated according to Hess ('27).

Vitamin depletion, as measured by eye condition and more particularly by body weight change, occurred in the average time of 26 days. Whenever it was determined that an animal

was definitely depleted, he was given the full amount of vitamin employed in the experiments (1450 or 2175 mg. of yellow corn) daily for 4 days to prevent the rapid onset of irreparable tissue damage. The definite but temporary effect of this treatment is clearly evident in the growth graphs of the negative control animals (chart 1).

Conduct of experiments. To eight groups of animals, following the period of readjustment described above, were administered respectively the following daily (note below the Sunday exception in the case of the mineral oil): no corn; 725 mg. of corn; 1450 mg. of corn; 1450 mg. of corn and 0.5 cc. of mineral oil no. 1; 1450 mg. of corn and 0.5 cc. of mineral oil no. 2; 2175 mg. of corn; 2175 mg. of corn and 0.5 cc. of mineral oil no. 1; 2175 mg. of corn and 0.5 cc. of mineral oil no. 2. These regimes were continued, if the animals survived, for 75 days. The animals were weighed and the food consumption measured twice a week. Body weight, eye condition and necropsy findings were the principal criteria of vitamin A economy.

The preparation, dosage and administration of the corn and mineral oil. The corn employed was the Improved Leaming Yellow Dent variety of seed quality. The same lot was used throughout the investigation. Although one method of preparing corn for assay is to weigh and sort the individual grains, we found it a better procedure for our purpose to employ the corn in the form of a homogeneous meal from which any desired quantity could be measured. A fresh supply was ground every 2 weeks and kept in the dark at a temperature of about 5°C. Although Marcus ('31) has shown that vitamin A concentrates undergo marked deterioration when dispersed on finely divided particles of various substances, Kick and Bethke ('29) found little loss in the vitamin A content of ground, whole or cracked yellow corn when stored for as long as 1 year. Preliminary assay of the corn was performed (compare groups I, II, III and VI in chart 2), and 1450 mg. chosen as the minimal daily amount which would permit a depleted animal to grow at approximately a normal

rate with disappearance of symptoms of vitamin A deficiency. The vitamin A of this corn apparently falls within the potency range reported in the literature (compare, e.g., Steenbock and Coward, '27, and Meyer and Hetler, '29). Comparison of groups III and VI of chart 2 indicates that 1450 mg. of corn is perhaps slightly less than the amount necessary for maximal growth under our experimental conditions. The corn was measured with an accuracy of ± 3 per cent.

Two commonly used standard brands of mineral oil, called mineral oils no. 1 and no. 2, were employed. Five-tenths cubic centimeter of oil comparable to a therapeutic dose for man was the daily amount administered to the rats. A short description of the oils and the reasons for the selection of

EXPLANATION OF CHARTS 1 AND 2

The growth curves depicted are of two kinds: chart 1, representative curves plotted in detail to serve as protocols, and chart 2, straight-line graphs to facilitate group comparison. The latter were prepared by drawing lines from an arbitrary common origin representing 40 gm. to points representing the final weights (in grams) attained. The corresponding abscissae represent the average depletion time, 26 days, plus the recuperation interval, 4 days (see text), plus the experimental period, 75 days; a total of 105 days. The black columns show averages of group food consumptions in grams for the experimental period. Of the two arrows shown in the protocol curves, the first records the time when the animal was adjudged to be definitely depleted of vitamin A; the second, the end of the 4-day readjustment period.

The Roman numerals indicate groups of animals receiving yellow corn and mineral oil in daily dosages as follows:

- I. No corn.
- II. 725 mg. of corn.
- III. 1450 mg. of corn.
- IV. 1450 mg of corn and 0.5 cc. of mineral oil no. 1.
- V. 1450 mg. of corn and 0.5 cc of mineral oil no 2.
- VI. 2175 mg. of corn.
- VII. 2175 mg of corn and 0.5 cc of mineral oil no 1.
- VIII. 2175 mg. of corn and 0.5 cc of mineral oil no. 2.

The cross designates death before the termination of the experimental period of 75 days. Other symbols adjacent to the ends of the curves indicate necropsy findings:

- E Definite ophthalmia.
- T. Abscess formation in the tongue.
- S. Abscess formation in the submaxillary glands
- K Calculi in the kidneys.
- B. Calculi in the urinary bladder.

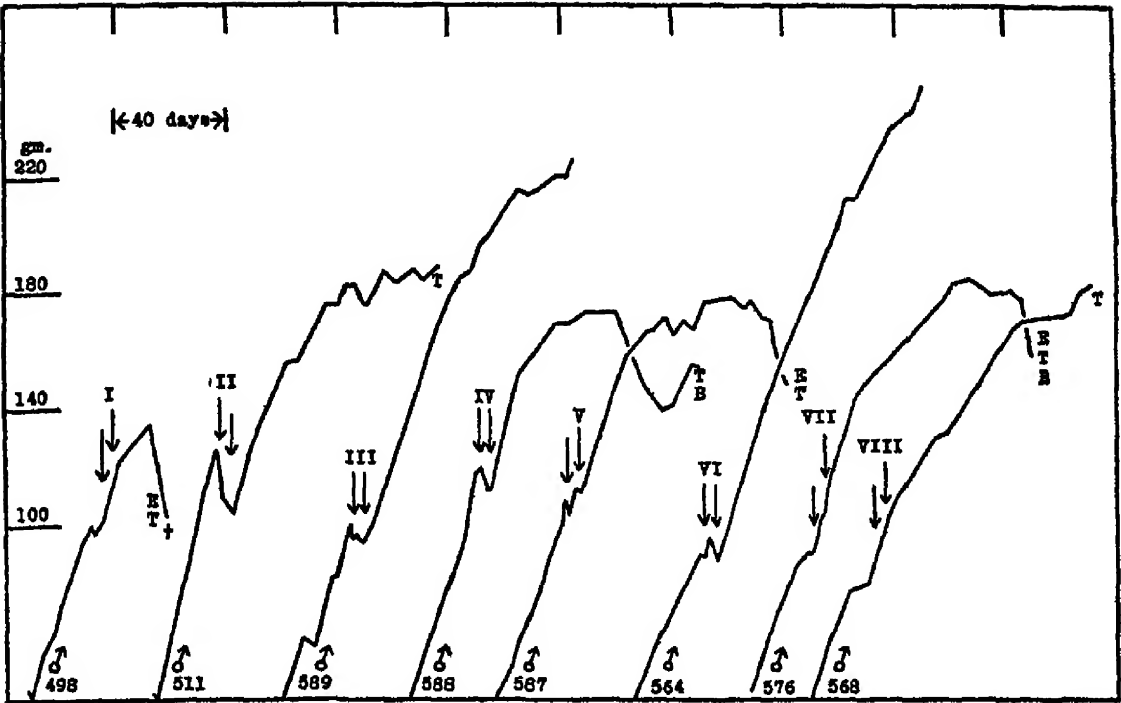


Chart 1

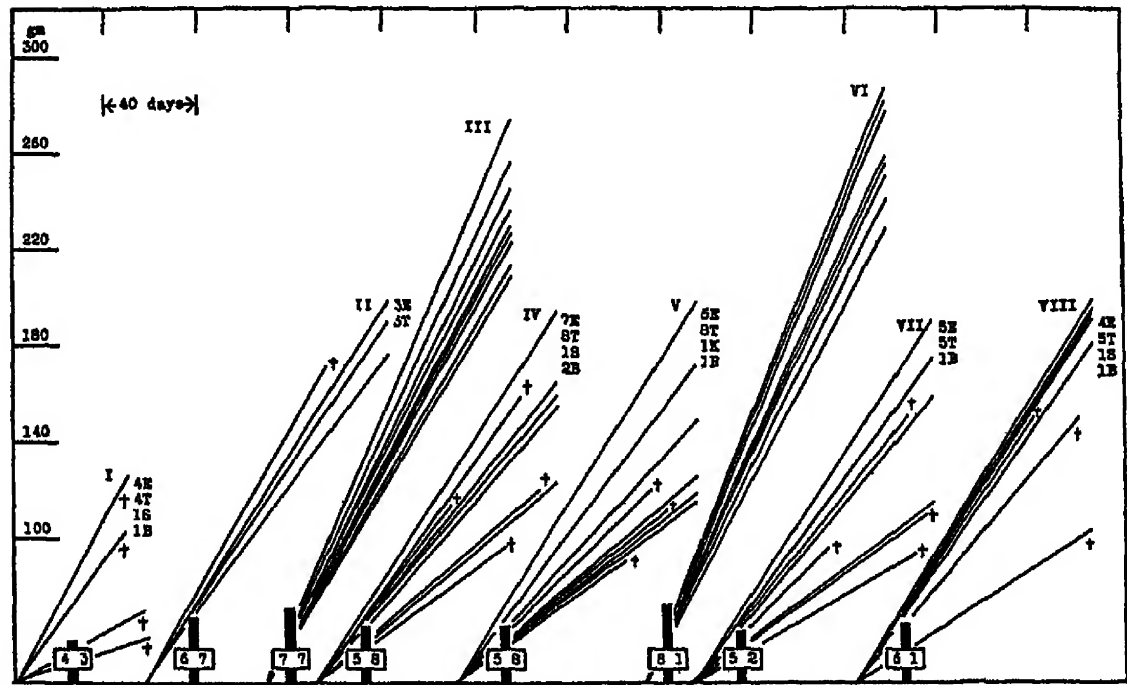


Chart 2

this dosage have been given previously and need not be rehearsed here. It is of interest, however, to note that Rowntree ('31) likewise estimated that 0.5 cc. of mineral oil 'approximates two tablespoons for the human being' and employed experimental doses from 0.25 to 0.75 cc. The 0.5 cc. of oil administered daily to our rats did not produce any degree of diarrhea. However, the oil was responsible, as shown by the time necessary for the elimination of markers, for a definite but rather moderate acceleration of intestinal motility.

The mineral oil was administered—separately from the corn—by means of a 1-cc. insulin syringe equipped with a clean glass tube for each delivery. The schedule for the giving of mineral oil was as follows: 0.17 cc. daily except Sunday at 8 to 9 A.M., 2 to 3 P.M. and 5 to 6 P.M. The corn was dispensed daily at 11 to 12 A.M. The corn was not ingested avidly as was the butter fat, but ordinarily disappeared during a period of 3 hours and was not left over to the following day.

DISCUSSION OF RESULTS

Charts 1 and 2 show clearly that administration of mineral oil seriously interferes with the animals' utilization of the vitamin A of yellow corn. This is true whether the corn was employed in a marginal intake of 1450 mg. or in one 50 per cent larger, 2175 mg. A similar finding was secured with a series of female rats (growth graphs not included here) which received 1450 mg. of corn and 0.5 cc. of mineral oil. These results are quite in contrast to those of the butter-fat studies which showed that mineral oil administered separately diminished only slightly the effect of a marginal dose of 100 mg. of butter fat and apparently not at all that of 150 mg. of butter fat.

Inasmuch as the corn was not eaten immediately, as was the butter fat, a series of male animals was depleted of vitamin A and then given the 0.5 cc. of mineral oil in three doses at 8 to 9 A.M., 11 to 12 A.M. and 2 to 3 P.M. and the 2175 mg. of

yellow corn at 5 to 6 P.M. Thus the last dose of mineral oil was administered 3 hours before the corn with a 14-hour period following before the next dose of mineral oil. These measures, instituted to insure a better separation of the corn and mineral oil in the gut, did not alter the outcome of the tests. The growth graphs are very similar to those depicted in groups VII and VIII in charts 1 and 2.

One suggestion growing out of these experiments was that the vitamin A potencies of the corn and the butter fat were due to two different substances with different properties. Recently, Dutcher, Harris and Guerrant ('33) have projected such a conception based on the discoveries of the last few years concerning the natures and physiological relationships of carotene and vitamin A and explain somewhat similar results of theirs as follows:

In a previous publication from this laboratory, it was shown that rats fail to show typical vitamin A response when butter fat is fed in the presence of mineral oil. Vitamin A in cod liver oil, however, is used quite satisfactorily under similar experimental conditions. The present experiments offer a possible explanation for the results just described. Carotene (dissolved) in corn oil is not utilized in the presence of mineral oil, while a carefully prepared pigment-free vitamin A concentrate (from cod liver oil) is utilized readily when fed with mineral oil. The hypothesis is advanced that these results are due to preferential solubility of hydrocarbon (carotene) in hydrocarbon (mineral oil) and the preferential solubility of sterol (vitamin A) in the sterol containing secretions of the intestinal tract.

However, Baumann and Steenbock ('33) have recently reported that: "In the sum total of biological activity (of butter) carotene is not of major importance, since it accounted for only 15 per cent of the total." It appears, therefore, that the hypothesis of Dutcher, Harris and Guerrant could apply to only 15 per cent of the butter-fat potency, i.e., the carotene portion. These writers do not state whether their pigment-free vitamin A concentrate and carotene were fed under the comparable condition of approximately equivalent dosage.

Different writers have made the reasonable observation that the amount of vitamin A fed is an important factor in determining the effect of the mineral oil. In other words, a sufficient excess of the vitamin seems to surmount the effect of the mineral oil. At all events, their hypothesis itself appears to be supported by our experiments wherein the mineral oil interfered with the utilization of the vitamin A factor to a much greater degree in the case of the corn than in that of the butter fat. Butter fat, according to Baumann and Steenbock, is 85 per cent vitamin A,² while the vitamin potency of the yellow corn like that of other plant products is presumably due to the presence of carotene.

The experiments herewith described showing that the separate administration of mineral oil distinctly inhibits the assimilation of the vitamin A factor in yellow corn do not favor the indiscriminate use of mineral oil as a laxative. On the other hand, it is to be noted that approximately one-third to one-half of the vitamin served its purpose and that, moreover, a varied diet supplies, in addition to carotene, vitamin A which apparently is better utilized in the presence of mineral oil. Furthermore, although relatively large doses of the corn were not tested, it seems generally possible in this type of experiment to overcome the effect of the mineral oil by the inclusion of extra vitamin A in the diet.

SUMMARY

1. The administration to albino rats of mineral oil separately from restricted doses of yellow corn interferes considerably with the assimilation of the vitamin A factor in the corn.

2. A discussion of the different responses produced by the vitamin A factors in butter fat and yellow corn is presented.

² More recently, Shrewsbury and Kraybill ('33) have concluded that: "The carotene of butter may account for an appreciable amount of its vitamin A activity."

ADDENDUM

The reader's attention is directed to a recent publication by H. S. Mitchell ('33), "Influence of mineral oil on assimilation of vitamin A from spinach," *Proc. Soc. Exp. Biol. and Med.*, vol. 31, p. 231. Mitchell's results with spinach are very similar to the writer's with yellow corn and are in accord with the thesis discussed in this paper that mineral oil in the gut exerts a much more marked influence on the assimilation of carotene than on that of vitamin A.

CORRECTION

In the first paper of this series (Jackson, '31), group VII of chart 5 on page 181 should bear the legend: 5E, 4T, 1B—comparable to similar legends accompanying the other groups.

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THE EFFECT OF MINERAL OIL ADMINISTRATION UPON THE NUTRITIONAL ECONOMY OF FAT-SOLUBLE VITAMINS

III. STUDIES WITH VITAMIN D OF IRRADIATED ERGOSTEROL¹

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Mineral oil in the gut may compete with natural fat as a solvent for fat-soluble substances. The fraction retained by the mineral oil which is itself not absorbed to any appreciable degree can be expected to escape the body. These considerations have been shown to have an important bearing relative to the effect of the ingestion of mineral oil upon the bodily economy of the fat-soluble vitamins. In addition to a considerable literature dealing with vitamin A in this regard, a few similar contributions on vitamin D have appeared.

Györky ('27) pointed out that olive oil should prove better than paraffin oil as a diluent for irradiated ergosterol because olive oil unlike mineral oil is absorbed. Nevertheless, Dutcher, Ely and Honeywell ('27) reported that cod liver oil diluted with mineral oil promotes calcification in rachitic rats to the same degree as an equal amount of cod liver oil without the mineral oil. The daily dose of cod liver oil was 4 drops (118 mg); of the cod liver oil-mineral oil mixture, 15 drops containing 4 drops of the former. Indices of the alleviation of rickets were bone ash percentage and the 'line' test.

On the other hand, Hawk, Levine, Stuckey and Oser ('29) state:

¹The expenses of this investigation were defrayed from the income of the Mineral Oil Fellowship, Yale University, 1929-1930.

Using the 'Line Test' and X-ray as criteria, it was demonstrated, both in preventive and curative experiments, that mineral oil also interfered with the utilization of vitamin D in cod liver oil. Approximately three to four times as much vitamin D was required to produce recalcification when mineral oil, as a diluent, was substituted for cottonseed oil.

The different results obtained by Dutcher, Ely and Honeywell and Hawk, Levine, Stuckey and Oser are apparently to be explained on the basis of the smaller dosage (2 to 12 mg.) of cod liver oil employed by the latter authors.

The experiments cited above deal with the effect of mineral oil given in an intimate mixture with the material containing the vitamin D. The following study was undertaken to determine the influence of mineral oil administered separately from the vitamin D. The experiments were thus designed to simulate the customary human practice in the use of mineral oil.

EXPERIMENTAL PART

Animals, diet and feeding technic. Albino rats² approximately 4 weeks of age and weighing from 50 to 60 gm. were confined four to a cage and fed a high calcium-low phosphorus rachitogenic diet for 24 days. Growth records were kept during this time. The animals were then recaged singly and divided into groups which were subjected to various regimes for an experimental period of 15 days. Body-weight and food-consumption records were kept for this period. Starvation was thereby demonstrated not to be a factor in the healing of the induced rickets. Fat was sometimes incorporated to make the diet more nearly like the average food of man, but the amount was restricted to avoid a correction of the abnormal calcium-phosphorus ratio in the gut (compare Zucker and Barnett, '22-'23). All diets were fed ad lib.

The irradiated ergosterol³ preparations employed were made up in and diluted with corn oil⁴ to give the proper dos-

² The animals employed in experiment 1 were reared on Sherman's diet B; those in experiment 2 on a calf meal ration (Maynard, '30). Both diets were somewhat modified for our use.

³ The writer wishes to thank Dr. Charles N. Frey, of the Fleischmann Laboratories, New York, for the ergosterol preparations.

⁴ Mazola.

ages in 20-mg. drops which were administered daily at 11 to 12 A.M. by means of a calibrated medicine dropper. The mineral oil, of two kinds, no. 1 and no. 2, previously described (Jackson, '31) was given daily in three equal portions at 8 to 9 A.M., 2 to 3 P.M. and 5 to 6 P.M. by means of an insulin syringe.

At the conclusion of the experimental period, the animals were killed and the femora and either the tibiae or the radii and ulnae were removed. The femora from each rat were carefully freed of adhering tissue, and the ash determined as per cent of dry fat-free substance according to the directions of Shohl and Bennett ('27). The other bones were subjected to the 'line' test with silver nitrate (McCollum, Simmonds, Shipley and Park, '22, and Coward, '28) or were decalcified in Müller's fluid, sectioned and stained with hematoxylin and eosin for histological examination.

Experiment 1

Both male and female animals were employed. Steenbock's diet 2965 (Steenbock and Black, '25) plus 10 per cent by weight of hydrogenated vegetable oil⁵ was fed throughout. The average gain in body weight for the 15-day experimental period was 5 gm. The amount (0.005 mg.) of viosterol employed in the mineral oil tests was in excess of the minimum required to induce a considerable recalcification. The total daily dosage of mineral oil was 0.5 cc.

Experiment 2

Only male albino rats were employed. Diet 2965 was fed for the initial 24 days, and then diet 2965 with 5 per cent of added hydrogenated vegetable oil for the experimental period of 15 days. The animals on the average just maintained their body weights during this latter period. To exclude the effect of laboratory light (compare Dutcher and Honeywell, '29), the animals were maintained for the entire 39 days behind a

⁵ Crisco.

black curtain. The irradiated ergosterol preparation was a different one than that used in experiment 1; the amount (0.001 mg.) employed in the mineral oil tests was insufficient to promote more than a partial calcification. The total daily dosage of mineral oil was 0.3 cc.

DISCUSSION OF RESULTS

The values for bone ash presented in table 1, experiment 1, exhibit no significant differences for groups II to V, although

TABLE 1

*The calcifying effect of irradiated ergosterol with and without mineral oil.
Experiment 1*

GROUP NO.	NUMBER OF ANIMALS	DAILY DOSE OF IRRADIATED ERGOSTEROL	DAILY DOSE OF MINERAL OIL	AVERAGE DAILY FOOD CONSUMPTION	BONE ASH, ON THE BASIS OF DRY FAT-FREE SUBSTANCE		
					Lowest value	Highest value	Average value
I	6	mg. None	cc. None	gm. 6.2	Per cent 26.8	Per cent 36.9	Per cent 33.1
II	7	0.001	None	5.8	34.2	46.0	40.0
III	8	0.005	None	5.2	36.3	43.7	40.0
IV	8	0.005	0.5 of no. 1	5.2	38.4	45.0	42.0
V	8	0.005	0.5 of no. 2	5.2	37.4	44.6	41.5

the average figure for group I receiving no irradiated ergosterol is considerably lower—33.1 per cent. 'Line' tests performed on the radii and ulnae revealed a similar relation. The animals of group I were shown to have marked rickets but those of the remaining groups were found to present pictures of advanced healing. Thus, administration of 0.5 cc. of mineral oil daily to the animals was without apparent effect on the utilization of the vitamin D; however, the dosage of the latter was at least five times the amount required to induce the maximum effects observed.

In experiment 2 a different specimen of irradiated ergosterol was examined under somewhat altered conditions as previously described. One of these conditions was that of administering a sub-marginal dose of irradiated ergosterol; another, that of keeping the animals in the dark. The average value for the bone ash of the femora of the negative control animals (group I) is 25.3 per cent. The other groups exhibit a progressive elevation of the bone ash with increase in the dosage of the irradiated ergosterol. The administration of mineral oil is without any apparent effect on calcification as

TABLE 2
*The calcifying effect of irradiated ergosterol with and without mineral oil.
Experiment 2*

GROUP NO.	NUMBER OF ANIMALS	DAILY DOSE OF IRRADIATED ERGOSTEROL	DAILY DOSE OF MINERAL OIL	AVERAGE DAILY FOOD CONSUMPTION	BONE ASH, ON THE BASIS OF DRY FAT-FREE SUBSTANCE		
					Lowest value	Highest value	Average value
I	9	mg None	cc. None	gm. 4.8	Per cent 24.2	Per cent 28.1	Per cent 25.3
II	8	0.0002	None	5.5	28.6	33.5	30.8
III	10	0.001	None	5.0	29.7	37.6	32.9
IV	10	0.001	0.3 of no. 1	5.3	28.4	36.5	32.9
V	10	0.001	0.3 of no. 2	5.4	29.4	35.8	33.1
VI	8	0.005	None	5.7	32.2	38.4	35.3

measured by ash determination, all the averages for groups III, IV and V being very close to 33 per cent. Sections of the tibiae were stained with hematoxylin and eosin and examined under the microscope.⁶ The negative controls (group I) uniformly revealed the development of severe rickets without healing. Half of the animals of group II showed no healing and half, from 1 plus to 2 plus healing. Five of the seven animals of group VI showed a nearly normal structure. The

⁶ The writer expresses his appreciation to Miss Deborah Jackson of the Department of Pediatrics of the Johns Hopkins Hospital for the examination of these sections.

members of groups III, IV and V were intermediate in this respect; approximately half in each series showed no healing and half from 2 plus to 3 plus healing.

These results show no adverse effect upon calcification when mineral oil is administered separately from the oil bearing the vitamin D. Furthermore the comparable nutritive state of the experimental animals of the mineral oil groups and the corresponding control group in each experiment is indicated by the nearly identical food consumptions.

SUMMARY

Mineral oil administered separately from irradiated ergosterol was not found to interfere with the utilization of vitamin D.

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THE CHEMISTRY OF THE BLOOD OF NORMAL CHICKENS

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In an investigation of the effect of the Rous sarcoma no. 1 upon the chemistry of the blood of hens (Roe and Dyer, '30, '31; Dyer and Roe, '32), it was necessary to establish normal values by determining the constituents of the blood of control hens. Since the literature contains very little information upon the blood chemistry of hens and hens are used rather extensively in certain types of experimental work, the authors feel that a published summary of their findings will be of value. In this paper we are reporting a summary of chemical analyses of normal hens' blood which includes values for twenty-three constituents. The reference to the method of analysis used, the number of hens, the number of determinations, the lowest and the highest individual values, and the average value for each determination are given.

The hens used were of the Barred Plymouth Rock strain and were obtained from market stock. Their average weight was approximately 2 kg. They were kept on a diet of water and 'scratch feed,' which is a commercial preparation of cracked corn, wheat, barley, buckwheat, milo maize, and sunflower seed. All analyses were performed after the hens had been fasted for approximately 24 hours.

The methods of analysis were thoroughly tested and sufficient analytical skill to obtain reproducible results was developed before undertaking the study of a blood constituent. Because of the undesirable influence of excessive bleeding,

The chemistry of the blood of normal hens (values are expressed as milligrams per 100 cc of whole blood unless otherwise indicated)

CONSTITUENT	NUMBER OF HENS	NUMBER OF DETERMINATIONS	VALUES			METHOD OF ANALYSIS
			Lowest	Highest	Average	
Non-protein nitrogen	13	13	28	47	36	Dupray's modification of Folin and Wu ('27)
Urea	4	10	2.1	5.4	3.3	Leiboff and Kahn ('29)
Uric acid	9	9	2.6	7.5	4.2	Folin's direct ('22)
Creatinine	17	17	0.8	1.5	1.3	Folin and Wu ('19)
Albumins of plasma	8	22	870	1,970	1480	Greenberg ('29)
Globulins of plasma	8	22	1460	2,770	1830	Greenberg ('29)
Hemoglobin	5	5	6300	11,000	8800	Van Slyke and Stadie ('21)
Sugar	19	19	127	190	157	Rothberg-Evans modification of Folin and Wu ('23)
CO ₂ -combining power ¹	10	10	24	53	42	Van Slyke ('17)
pH	14	72	7.22	7.45	7.36	Hastings and Sendroy ('24)
Fatty acids	8	14	411	1,560	847	Bloor, Pelkan and Allen ('22)
Carotin of plasma	6	10	0.29	2.0	0.99	Connor ('28)
Cholesterol	15	15	114	244	168	Bloor ('16)
Phospholipids (as lecithin)	10	15	231	580	420	Modification of Fiske and Subbarow ('25)
Phosphorus (inorganic of serum)	6	6	2.7	5.9	4.6	Modification of Fiske and Subbarow ('25)
Phosphorus (total of plasma)	4	19	10	19	13	Modification of Fiske and Subbarow ('25)
Phosphorus (total)	9	38	92	132	105	Modification of Fiske and Subbarow ('25)
Chlorides as NaCl	14	14	517	644	563	Modified Whitehorn ('21)
Sodium of serum	10	18	279	385	345	Kramer and Gittleman ('24)
Potassium of serum	9	17	19	27	22	Breh and Gaebler ('30)
Potassium	7	13	130	192	164	Breh and Gaebler ('30)
Calcium of serum	4	4	12.4	12.8	12.7	Roe and Kahn ('29)
Magnesium of serum	5	12	1.6	3.0	2.4	Eichholtz and Berg modification of Yoshimatsu ('30)
Glutathione (reduced)	9	14	60	94	75	Hess ('29)
Bilirubin of serum	4	4	Trace	Trace	Trace	Van den Bergh ('16)

¹ Volumes per cent.

it was decided not to make duplicate determinations of all constituents, and accordingly duplicate determinations were made only upon those constituents for which we felt the method of analysis was not sufficiently accurate to give entirely satisfactory results by single determinations. Duplicate determinations were made in estimating the non-protein nitrogen, fatty acids, total phosphorus, phospholipids, calcium, magnesium, sodium, potassium, albumins and globulins. In view of the fact that a great number of determinations was made we believe that our findings represent true values for chickens' blood.

Values for some of the constituents in our summary have been reported by Hayden and Fish ('28), Hughes, Titus and Smits ('27), Haam and Stöhr ('30), Holy ('30), Moravek ('32, '32 a), and Kobliha ('30). The reports of these authors are in agreement with our findings when the variations due to the methods of analysis used are taken into consideration.

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THE RELATION OF FOOD TO ALIMENTARY FILL IN THE ALBINO RAT ¹

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It is generally recognized that the basal heat production of animals differing in size is not a constant, in relation to their respective body weights; consequently diverse conclusions have arisen as to the relationship of body size to metabolism, and various units of reference for basal metabolism have been suggested.

Inasmuch as any one of these units ultimately involves the weight of the animal, it is immaterial, for the purpose of the present consideration, whether heat production is referred to body weight directly, or to some functional power of the body weight, or to derived units, such as computed body surface, or active protoplasmic mass. It is of interest, however, to consider whether there is not a more significant measure of body weight than the gross weight, as a unit of reference for metabolism, or as a basis for the computation or derivation of units of reference.

The degree of fatness of an animal is one factor which influences the extent of the body surface, and of the body weight—in a sense 'diluting' the active body tissue participating in metabolism (Moulton, '16).

Another factor, which was considered by Rubner (1883), is the content of the alimentary tract, which represents a considerable and a varying proportion of the body weight, but

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does not constitute a direct or an immediate proportionate cause or source of heat production.

The present paper presents certain observed relationships between the quantities of the food eaten and of the alimentary fill, and calls attention to their significance in the statement of results of metabolic research.

EXPERIMENTAL

This study of fill was conducted on a separate group of animals in connection with a concurrent investigation of the influence of the plane of nutrition on the heat production and on the specific dynamic effects of nutrients, the two groups of animals, which were albino rats weighing about 100 gm. each, being fed and managed alike. The dietary treatment is given in table 1.

The rats were confined in individual cages with false screen bottoms to lessen coprophagy. The daily food was given in two equal portions, at 8 a.m. and 4:30 p.m.

In the energy metabolism studies the following procedure was followed: With all except the fasting rats the metabolism experiments were conducted after the subjects had been not less than 3 days on a specific dietary treatment. The experiments were started immediately after the morning feed allotment had been consumed—which was usually within an hour after feeding. With fasting rats a maintenance allowance of a complete diet was fed for 3 or more days. The animals were then used for the metabolism experiment 24 hours after receiving the last half-daily portion of feed.

In the concurrent study of fill the rats were killed after the same time following feeding as elapsed with the rats used for metabolism studies before respiration measurements were begun. The rats were killed by asphyxiation, and were weighed on a large analytical balance. The alimentary tracts were removed and emptied, and the contents were placed in weighing bottles, and weighed. At least five individuals were used for each dietary treatment. The results are presented in table 1.

TABLE 1
The relation of the quantity of feed to the quantity of the contents of the alimentary tract

GROUP	NUMBER OF ANIMALS	DAILY DIETARY TREATMENT	WEIGHT OF CONTENTS OF ALIMENTARY TRACT EXPRESSED AS PER CENT OF GROSS BODY WEIGHT								Mean
			Individual determinations								
1	5	Fasting	2.39	2.73	3.02	3.36	3.87	3.07 ± 0.18	
2	5	1 56 gm. olive oil	4.70	5.05	5.68	5.77	5.88	5.42 ± 0.15	
3	5	3 80 gm. casein	4.84	5.39	6.52	6.92	7.02	6.14 ± 0.29	
4	6	3 92 gm. corn starch	4.63	5.69	6.66	7.49	7.58	8.14	8.14	6.70 ± 0.35	
5	5	4.00 gm. calf meal ¹	8.88	9.21	9.26	9.69	10.07	9.42 ± 0.14	
6	5	5.50 gm calf meal	9.57	9.58	9.87	10.47	10.63	10.02 ± 0.15	
7	5	5 50 gm. calf meal + 1 gm. olive oil	9.04	9.28	9.44	9.45	9.86	.	.	9.41 ± 0.09	
8	5	5 50 gm calf meal + 2.0 gm casein	8.56	9.83	10.20	10.53	10.84	9.99 ± 0.29	
9	5	5 50 gm. calf meal + 2.2 gm. corn starch	9.89	9.99	10.18	10.83	10.88	10.35 ± 0.14	
10	5	8 00 gm. calf meal	8.52	11.24	11.52	11.63	13.38	11.26 ± 0.53	

¹The calf meal diet was composed of 300 parts linseed oil meal, 400 parts corn meal, 200 parts ground malted barley, 440 parts red dog flour, 240 parts dried skim milk, 300 parts oat flour, 60 parts soluble blood meal, 20 parts salt, 20 parts ground limestone and 20 parts steamed bone meal

DISCUSSION

The energy requirement for the maintenance of a 100-gm. rat was approximately satisfied by the olive oil, starch and casein fed to groups 2, 3 and 4, and by the 5.5 gm. allowance of calf meal fed to group 6. Groups 7, 8, 9 and 10 received food in excess of the amounts required for maintenance.

The lowest proportion of fill, 3.07 per cent, was obtained from the fasting rats. For the groups fed the approximate requirements of maintenance—the fill with olive oil was 5.42 per cent; with casein, and with starch, 6.14 and 6.70 per cent, respectively; and with 5.5 gm. of calf meal, 10.02 per cent. The fill with 4 gm. of calf meal was considerably greater than with almost equal quantities of starch or casein, and was only slightly less than with 5.5 gm. of calf meal. These figures indicate that the proportion of fill is influenced both by the quantity and by the character of the feed.

The addition of casein or starch to the maintenance diet of calf meal did not result in a significant increase in fill. The fill on the maintenance diet of calf meal plus olive oil was slightly lower than that for the same quantity of calf meal alone—probably because of a laxative effect of the olive oil.

An increase in the quantity of calf meal from 5.5 gm. to 8 gm., that is, a 45 per cent increase in the diet, resulted in a 12 per cent increase in fill, and affected the weight of the animal by only 1.2 per cent. In other words at the super-maintenance levels the influence of the character of the diet on the fill was much less pronounced than at the maintenance levels. This was, of course, partly due to the fact that the greater part of all the supermaintenance rations here considered consisted of the same calf meal, whereas the maintenance rations differed much in character. The fact, however, that the addition of the various single supplements, and of more calf meal, to the maintenance ration of calf meal did not result in any considerable increase in fill signifies that with increase in food, of any kind, there is a proportionately greater increase in the rate of passage of food residues through the alimentary tract.

The foregoing observations are especially important in relation to the comparison of the rates of metabolism of animals on different dietary treatments. From unpublished results of metabolism experiments the authors have computed the daily heat production of fasting rats, and of rats fed a maintenance quantity of a complete diet, i.e., 5.5 gm. per day. When the heat production is expressed per 100 gm. of empty body weight, the heat increment resulting from the 5.5 gm. of feed is 3360 small calories. If no correction is made for the alimentary fill the heat increment is 2808 calories. A difference of the same order obviously results if other units of reference for metabolism are used, the derivation of which involves the body weight; for instance, computed body surface.

The individual variation in the fill of animals on the same dietary treatment was slight.

In harmony with this finding Trowbridge, Moulton and Haigh ('15) observed average variations in the fill of mature beef cattle of only 2 to 4 per cent of the live weight, and suggest that the fill can be disregarded in many cases with those animals. However, it is more logical to relate the metabolism to a body weight which represents only body tissue; and such procedure is important for a close comparison of the metabolism of animals subjected to different dietary treatments.

SUMMARY

The quantity of the alimentary fill, expressed as a percentage of the live weight, has been determined with ten groups of albino rats, one group being fasted, the other groups being given different dietary treatments.

The ratio of fill to gross body weight was found to be somewhat affected by both the kind and amount of feed, such effects being more prominent at the maintenance level than at higher planes of nutrition.

The results indicate that with increase in food there is a proportionately greater increase in the rate of passage of food residues through the alimentary tract.

The alimentary fill of rats which received food was greater than that of fasting rats by 2.4 to 8.2 per cent of the gross body weight, in accord with the dietary treatment.

It is therefore important to consider alimentary fill when comparing the metabolism of animals subjected to different dietary treatments.

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A COMPARISON OF EXTRACTION METHODS USED FOR VITAMIN A DEPLETION DIETS FOR RATS

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INTRODUCTION

The present-day worker in the field of vitamin A assay is confronted with many problems in determining the technic to use in his work. Osborne and Mendel ('13) and McCollum and Davis ('13) called attention to the varying length of time that different animals of the same species continued to grow upon diets lacking in vitamin A. Chick ('20) stated that in her work extending over several years there was a great deal of unexplained irregularity in the behavior of the stock rats when placed upon diets deprived of fat-soluble vitamins. Sherman and Boynton ('25) and Sherman and Storm ('25) found a wide variation in the survival period. The same observation was made by Sherman and Cammack ('26) the following year. The recent work of Sampson and Korenchevsky ('32) showed that there was a wide variation in the time required for rats placed upon a vitamin A-free diet to develop either xerophthalmia or loss of appetite.

Bacharach ('33) in his investigation into vitamin A-free basal diets said that many animals will continue growing on a basal diet for months with no evident signs of avitaminosis, although some animals on the same diet reach a plateau in 8 to 10 weeks. Other animals appear to stop growing for as long as 14 days and then begin to grow again for 4 weeks or more. He further stated that, "The literature abounds in

graphs showing animals that have been considered 'runout' when their growth has only slackened, without actually ceasing, for a few days."

There is no standardized method of extraction of the casein offered at present. The solvent and the method and length of extraction reported by different workers varies greatly. Coward, Key and Morgan ('28) discussed at length the relative potencies of the extracts of casein made by several methods and solvents. They found that the factor causing growth resumption is removed from casein only with difficulty. Insufficient purification of the basal diet was, in the opinion of Drummond and Coward ('20), responsible for many variations. They stated that their own experience had given many examples of the fact that the presence of very small amounts of the vitamin A factor in an insufficiently purified basal diet may confuse the issue of the experiment. An analysis of the technic of representative workers showed a wide variation in the solvent and method used. The time of extraction has varied from heating 1 week in shallow pans to 10 days' continuous extraction with alcohol in a percolator.

The problem of the purification of the basal diet is of considerable importance. The presence of even small amounts of vitamin A in this diet will result in a prolonged depletion period and will cause a higher vitamin value to be assigned to the food being assayed.

Most of the published work concerning vitamin A has appeared in papers dealing with the detection or estimation of this vitamin, while few studies of the method of purification of the vitamin A-free diet have been reported.

The purpose of this study was to compare the effect of different methods of extraction with 95 per cent alcohol on the removal of vitamin A from the casein used in basal diets for rats.

PROCEDURE

The rats used in this study were of the albino or pied variety of known nutritional history from the stock colony. The mothers of these rats had been reared on a diet consisting of:

	<i>Per cent</i>
Whole wheat ground fine	30
Yellow cornmeal	60
Linseed meal	4
Bran	4
Sodium chloride (NaCl)	1
Tri-calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$)	1

Cod liver oil ($\frac{1}{2}$ cc. per rat) was mixed with enough of the above for 1 day's feeding and water added to make a stiff paste. The diet was supplemented by milk and raw liver. The milk was fed ad libitum. The liver was ground and 2 to 3 gm. fed per rat per week.

One hundred and thirty rats were placed upon six different experimental diets. The general plan of the experimental work was to feed a diet nutritionally adequate except for vitamin A.

The vitamin A-free or depletion diet, a modification of Rowntree's ('29) basal diet, consisted of:

<i>Material</i>	<i>Grams</i>	<i>Remarks</i>
Casein	18	Commercial casein (crude Argentine ¹), extracted with hot alcohol (95 per cent by volume).
Yeast	10	Pure dehydrated yeast. ²
Salt mixture	4	McCollum's no. 185.
Cornstarch	68	Commercial cornstarch (unpurified).
Vioosterol	0.05	To the above was added 0.05 cc. of 2 per cent potassium iodide (KI).

¹ Atterbury Brothers, 145 Nassau Street, New York, N. Y.

² The Northwestern Yeast Company, Chicago, Illinois.

The animals were weighed weekly for the first 4 weeks, twice weekly thereafter until a stationary weight was reached, and then daily. They were examined for any outward signs of vitamin A depletion. A record of food consumption was kept for each cage of rats. At the end of the experimental period the rats were examined thoroughly, autopsied and a record made of their condition.

The casein used in diet I was extracted with hot alcohol for 16 continuous hours. The extraction was a modification of the Sperry method ('26) as used by Rowntree ('29). To 200 gm. of fine-grained, air-dry, crude Argentine casein were added 500 cc. of boiling 95 per cent alcohol and the mixture was allowed to stand overnight to prevent caking. The following day the material was filtered by suction in a Büchner filter. The casein was placed in a cheesecloth bag inside an enamel percolator. The alcohol was placed in a 2-liter Pyrex flask, and kept at a slow rolling boil. A round-bottom 2-liter Pyrex flask fitted tightly into the top of the percolator, and served as a condenser. At the end of the extraction period the hot casein was filtered rapidly by suction in a Büchner filter, air dried, and then heated for 1 week in shallow pans in an electric oven at 105° to 110°C. The material was stirred thoroughly each day.

In diet II the method of extraction of the casein was a combination of the Sperry method as described for diet I, and Sherman's method ('31). The same apparatus was used. The casein (200 gm.) was treated in three successive 1-hour extraction periods, using 500 cc. of fresh alcohol for each extraction. Finally, the casein was dried in the air at room temperature.

The method used for the extraction of the casein fed to rats on diet III was the same as that used for diet I with the exception that 1500 cc. of 95 per cent alcohol were used to 200 gm. of casein.

An entirely different technic was followed for the extraction of the casein for diets IV and V. The Sherman ('31) method was used. Two hundred grams of casein (without previous soaking) were placed in a 2-liter Pyrex flask with 500 cc. of 95 per cent alcohol. This was boiled 1 hour under a reflux condenser on an electric plate. The contents of the flask were transferred rapidly without cooling to the Büchner filter and the hot alcohol removed by suction. The casein was returned to the flask, 500 cc. of fresh 95 per cent alcohol added, and the extraction continued for another hour. The process

was repeated a third time. The casein was dried in the air at room temperature.

The majority of the workers in the field do not extract the yeast to free it of vitamin A, believing that the amount of A is too small to be a determining factor. However, Sherman and Smith ('31) recommend that each laboratory carefully test the yeast that it uses in vitamin A work. Osborne and Mendel ('20) state that heating yeast overnight in an electric oven at 110°C. and then boiling it three successive times with

TABLE 1
Summary of methods of extractions of casein with 95 per cent alcohol

DIET	METHOD		AMOUNT OF		LENGTH OF EXTRACTION	REMARKS
	Direct	Indirect	Casein	Alcohol		
I		Casein in bag in percolator	gm 200	cc 500	hours 16	Continuous
II		Casein in bag in percolator	200	1500	3	Successive, fresh alcohol added
III		Casein in bag in percolator	200	1500	16	Continuous
IV	Casein in flask		200	1500	3	Successive, fresh alcohol added
V	Casein in flask		200	1500	3	Successive, fresh alcohol added
VI		Casein in bag in percolator	200	1500	48	Continuous

absolute alcohol under a reflux condenser for 1 hour does not affect the potency of the vitamin B and G content.

In diet V both the casein and the yeast were extracted by the direct method used for the extraction of the casein in diet IV.

A continuous 48-hour extraction was made on the casein used in diet VI by the indirect method used in diet III.

Table 1 gives a summary of the extraction methods, proportion of ingredients, and length of time of extraction used in the six different diets.

The young rats received the regular stock diet from the time of weaning until they were placed on the various diets. Each litter was kept in a small round raised-bottom screen cage which prevented coprophagy. When they approached maturity the males and females were separated. The experimental period was 10 weeks.

RESULTS

In this paper we have presented data on the effectiveness of different methods of extraction for the removal of vitamin A from casein used in vitamin A-depletion diets.

TABLE 2
Comparison of initial age and weight with final weight

DIET	NUMBER OF		AGE IN DAYS		WEIGHT IN GRAMS			
	Rats	Litters	Initial		Initial		Final	
			Range	Average	Range	Average	Range	Average
I	37	6	24-28	26.2	33-43	38.5	150-228	185.5
II	25	5	22-23	22.2	31-39	35.3	134-222	174.5
III	15	6	24-28	26.4	27-44	34.8	97-149	123.2
IV	17	6	21-24	22.7	29-39	33.1	67-154	121.1
V	18	6	21-25	23.2	27-38	32.0	104-149	121.6
VI	18	6	22-28	25.8	27-38	34.6	93-185	145.1

The results from diets I and III have been compared to show the effect of varying the amount of alcohol; diets II and IV to determine the effect of different methods of extraction; diets III and VI to note the effect of a variation in the time of extraction; and diets IV to V to determine whether any appreciable amount of vitamin A could be removed from the yeast. Table 2 shows that there was very little variation in the initial ages and weights of the rats in the groups which were compared.

Diets I and III were the same in all respects except in the amount of alcohol used for the extraction of the casein. These two diets were studied to determine the effect of a different amount of alcohol on the removal of vitamin A from casein. Three times as much alcohol (1500 cc.) was used

in the extraction of the casein (200 gm.) for diet III as was used for diet I (500 cc.). Both extractions were made by the Sperry method for 16 continuous hours, placing the casein in a bag inside a percolator. Since all animals were bred on the same stock ration, and the average initial age was approximately equal, the principal conditions for the storage of vitamin A were constant. The results obtained from feeding these two diets were very different. No abnormal conditions were observed among the rats on diet I at autopsy. Ninety-four per cent of the rats on diet III developed lung infection, while 80 per cent showed an abnormal condition of the spleen. Thatcher and Sure ('32) stated that atrophy of the spleen

TABLE 3
Average gains per rat per week for experimental period

DIET	NUM- BER OF RATS	WEEK										AVER- AGE
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	
		<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
I	37	26.5	29.0	19.1	25.1	7.5	6.2	7.1	8.9	10.3	7.3	14.70
II	25	18.9	25.1	22.5	22.4	21.2	15.6	8.4	7.8	-0.4	-2.3	13.56
III	15	24.7	23.2	17.3	16.0	17.8	2.9	5.8	-2.9	-7.0	-9.4	8.84
IV	17	20.9	24.3	30.7	15.0	19.3	10.2	0.0	-9.9	-13.9	-14.0	8.26
V	18	18.4	24.6	22.6	23.2	16.9	10.6	1.0	-8.0	-11.5	-20.0	7.78
VI	18	18.3	24.9	19.5	22.4	15.0	9.3	4.8	-0.15	-1.1	-2.5	11.04

was a common finding in their vitamin A work. Nearly one-half gave evidence of an abnormal kidney condition, and about one-fifth showed abnormalities of the eye, ear and liver. The rats on diet I made a 381 per cent gain as compared with 254 per cent for those on diet III (table 3). The average weekly gain per rat was nearly twice as much on diet I as on diet III. The first break in weight on the two diets was at the same time (third week), but the final decline after which no further gain was made occurred in the seventh week in the rats on diet III, and in the tenth week on diet I. The daily food consumption was the same for the rats on both diets. Sixty-seven per cent of the animals on diet I showed a stationary or lowered weight for 3 successive days with a sub-

sequent gain, whereas only 53 per cent of the animals on diet III exhibited this phenomena. Bacharach ('33) stated that many animals on a vitamin A-free diet reached a plateau or showed a decline in weight for a few days and then continued to grow again for some time. Many workers in the field of vitamin A assay have used stationary weight for 3 successive days as the criteria for depletion. All the rats on both diets were alive at the end of the experimental period. The results obtained by feeding these two diets to rats indicated that the large amount of alcohol (1500 cc.) was more effective in the removal of vitamin A from casein (200 gm.) than the small amount (500 cc.).

Diets II and IV were compared to determine the effect of the method of extraction on the removal of vitamin A from casein. The casein in diet II was extracted by the Sperry method while Sherman's method was employed for the extraction in diet IV. The extraction was carried on with the same amount of alcohol (1500 cc.) and for the same length of time, 3 successive hours, with fresh alcohol each hour. No abnormal conditions were noticed among the rats on diet II. Fifty-eight per cent of the rats on diet IV developed a lung infection, while about one-half showed abnormalities of the ear and liver. In a few cases the pupil of the eye became white and opaque, resulting in apparent blindness. In some cases where inflammation of the lids was noticed among the rats in this group, the condition which is generally considered a sign of vitamin A depletion seemed to be due to colds and not to xerophthalmia. At various times rats from the stock colony suffering from colds have shown a more evident inflammation around the eye than was observed in this group of rats. One wonders whether some of the eye conditions which have been reported as xerophthalmia have been due merely to colds. It is difficult to distinguish between actual xerophthalmia in rats and the condition of the eyes commonly found in colds. The determination of the abnormality of the liver from general observation of a spotted condition is very subjective, and should be followed by a microscopical study if it is to be

considered a significant factor in depletion work. Slightly over one-fourth of the rats on diet IV developed an abnormal condition in the kidney, alimentary tract and nasal passages. Seventeen per cent of these animals had abscesses in the posterior portion of the tongue, while 5 per cent showed an abnormal eye condition and bloody urine. The rats on diet II made a significantly better per cent gain (394 per cent) for the entire experimental period than those on diet IV (239 per cent) (table 3). The average weekly gain was 13.92 gm. for the rats on diet II and 8.26 gm. for the rats on diet IV. The average daily food consumption for the two groups of rats was almost identical, varying only 0.5 gm. The first decline in growth for the rats on diet II was in the third week, and for diet IV in the fourth week. The beginning of the continuous decline occurred earlier in rats on diet II (third week) than in the rats on diet IV (sixth week). The weight of the rats on diet II held quite constant until the sixth week when an average loss of 15 gm. per rat occurred. Forty-eight per cent of the rats on diet II attained plateaus and later showed gains, whereas only 11 per cent did so on diet IV, indicating more uniform results for the latter diet. The lowest per cent of plateaus with subsequent growth was found in the rats on diet IV. Mortality among the rats on diet IV was 29 per cent. The method of boiling the alcohol and casein in the flask seemed to remove more vitamin A than the method of placing the casein in a bag in a percolator.

A comparison was made of diets IV and V to ascertain whether the yeast used in the six diets contained any appreciable amount of vitamin A which might confuse the results of an assay. The casein alone was extracted in diet IV, while both casein and yeast were extracted in diet V. The Sherman method was used for all extractions in these diets. There was approximately six times as great an incidence of abnormal eye condition in the rats on diet V. Forty-seven per cent of the rats on both diets developed pus in the ears. There were no instances of abscesses on the posterior portion of the tongue or of bloody nose and paws in rats on diet V, while

those on diet IV showed 17 and 27 per cent respectively of these conditions. A slightly higher incidence of lung, spleen, and kidney abnormalities appeared in the rats on diet V, while there was a slightly greater per cent of liver changes in the rats on diet IV. There was a marked difference in the condition of the alimentary tract at autopsy; the rats on diet V showed a higher per cent and greater severity of changes. A day or two before death the abdomens of the rats on diet V became swollen and hard, although excretion was normal. The tract was greatly distended with gas, somewhat darkened and in a few cases contained blood. Turner and Loew ('31), in their work on the infection of the accessory sinuses and upper respiratory tract in avitaminosis of rats, stated that at necropsy 40 per cent of the animals deprived of vitamin A showed a ballooning of the intestines and marked gastro-enteritis. While no abnormal condition of the urine was observed in the rats on diet V, 5 per cent of the rats on diet IV developed a bloody urine. Neither the average weekly gain nor the total per cent gain for the experimental period showed any significant variation in the rats on these two diets, which indicated that no appreciable destruction of vitamins B and G had resulted from the alcohol extraction of the yeast. These results seem to confirm the observations of Osborne and Mendel ('20) that the extraction of yeast with hot alcohol does not remove vitamins B and G. One-third of the rats on diet V reached plateaus and later gained in weight, while only one-tenth of the group did so on diet IV. The mortality of the rats was twice as high on diet V as on diet IV. The food consumption for the two diets varied 1.43 gm., which showed that there was no appreciable amount of the appetite factor removed by the extraction of the yeast. Since the general condition of the rats fed the extracted yeast was somewhat worse than the condition of the rats fed the unextracted yeast, it would seem to indicate that the yeast used in this study did contain a small amount of vitamin A. It is interesting to note that even though there were many differences between diet V and the other diets, the rats on diet V showed in general the worst condition.

Diets III and VI were comparable in all respects except the length of time of extraction of the casein. The Sperry method of placing the casein in a bag inside a percolator and allowing the alcohol vapors to pass up through the casein was used for the extraction in both diets. Fifteen hundred cubic centimeters of alcohol were used for the extractions in both diets. The casein in diet III was extracted for 16 continuous hours, whereas the extraction for diet VI was continued for 48 hours. The results from the two diets were quite similar. There was approximately the same per cent occurrence of abnormalities in the eye, ear, tongue, kidney and urine in

TABLE 4
Average daily food consumption per rat

DIET	WEEK										AVERAGE
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	
I	11.3	15.7	19.2	16.4	16.9	15.4	14.9	16.4	18.8	18.3	16.33
II	9.4	14.2	18.4	18.2	18.8	18.5	17.8	18.8	17.8	18.1	17.00
III	11.4	17.8	19.4	19.1	19.6	18.9	18.8	17.8	10.9	9.5	16.32
IV	9.5	15.6	19.2	22.7	20.9	19.6	19.8	16.7	11.5	9.1	16.46
V	9.9	14.1	19.6	19.6	19.1	18.8	17.1	13.6	11.8	6.7	15.03
VI	11.8	14.5	19.7	17.0	16.8	18.0	17.5	12.5	12.9	12.0	15.17

the rats on the two diets. Diet III showed a higher incidence of cases of bloody nose and paws, lung infection, liver and spleen changes. Eleven per cent of the rats on diet VI developed abnormalities of the alimentary tract, whereas none was evident in the rats on diet III. There was a considerably greater per cent gain (319 per cent) on diet VI than on diet III (254 per cent). The rats on diet III gained 8.84 gm. weekly compared to a gain of 11.04 gm. for diet VI. The difference of 1.75 gm. in weekly food intake was insignificant. There were no deaths among the rats on either diet. The results obtained from feeding diets III and VI indicated that a 48-hour continuous extraction was no more effective in the removal of vitamin A from casein than was an extraction period one-third as long.

Average food consumption, as shown in table 4, was practically equal in all the groups of animals with a maximum variation of 1.7 gm. per rat per week. The uniform food consumption of the rats on the six different diets shows that the differences in the results obtained were due to a deficiency

TABLE 5
Summary of results

REMARKS	DIETS					
	I	II	III	IV	V	VI
Number of rats on diet	37	25	15	17	18	18
Average initial agee (days)	26.2	22.2	26.4	22.7	23.2	25.8
Average initial weight (grams)	38.5	35.3	34.8	33.1	32.0	34.6
First decline in weight (weeks)	3rd	3rd	3rd	4th	4th	3rd
First continuous decline (weeks)	10th	3rd	8th	6th	5th	5th
Plateaus of weight— later gained	67%	48%	53%	11%	331/3%	55%
Per cent gain—10-week period	381	394	254	239	243	319
Average weekly gain (grams)	14.7	13.56	8.84	8.26	7.78	11.04
Average daily food intake (grams)	16.33	17.0	16.32	16.46	15.03	15.17
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Deaths	0	0	0	29	61	0
eye	0	0	20	5	33	16
ear	0	0	20	47	47	22
nose and paws	0	0	5	27	0	0
tongue	0	0	0	17	0	0
Abnormal condition of						
lungs	0	0	94	58	66	33
liver	0	0	13	41	38	5
spleen	0	0	80	35	47	55
kidney	0	0	40	29	33	42
tract	0	0	0	29	42	11
urine	0	0	0	5	0	0

in the diets, and not to a lowered food intake. The fact that the stock ration used for the colony was so high in vitamin A may explain in part the prolonged depletion which occurred in this study.

CONCLUSIONS

A comparison of the effect of different methods of extraction with 95 per cent alcohol upon the removal of vitamin A

from the casein used in depletion diets for rats under the conditions of this experiment showed that:

1. The large amount of alcohol (1500 cc.) was more effective in the removal of vitamin A from casein (200 gm.) than the small amount (500 cc.).

2. The method of boiling the alcohol and casein in the flask seemed to remove more vitamin A than the method of placing the casein in a bag in a percolator.

3. A 48-hour continuous extraction with hot alcohol was no more effective in the removal of vitamin A from casein than a 16-hour continuous extraction period.

4. a) A comparison of extracted and unextracted yeast used in this study showed that the sample of yeast tested contained a small amount of vitamin A.

b) Observations of the growth and food consumption of the rats on the extracted and unextracted yeast indicated no appreciable loss of vitamins B and G in the yeast extracted with hot 95 per cent alcohol.

5. The extraction of both the casein and the yeast by the Sherman method resulted in the removal of the maximum amount of vitamin A from the basal diet.

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VITAMIN C IN DELICIOUS APPLES BEFORE AND AFTER STORAGE¹

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TWO FIGURES

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The vitamin C potency of apples has been found to vary with the variety and with the length and mode of storage. It is of scientific interest, therefore, as well as of dietetic importance, to determine the vitamin C content of the different apple varieties and to ascertain the effect of storage upon the antiscorbutic properties of each important variety.

Experiments reported to date as summarized in table 1 indicate a wide range in the amount of apple required to prevent scurvy. The richest variety yet reported (Bracewell et al., '30, '31) is an English cooking apple, Bramley's Seedling, which protected guinea pigs from scurvy when fed in amounts as low as 3 gm. daily. For other English apples the minimum protective dose ranged from 10 gm. in three varieties to more than 20 gm. in three other varieties for which larger amounts were not tested. One variety furnished protection in 20 but not in 10-gm. amounts daily. The effect of storage was found by the English workers to differ, depending both upon the variety of apple and upon the temperature and composition of the atmosphere. Apples brought to England from other parts of the British Empire showed in general a lower vitamin C content than native apples of the same variety. This

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difference appeared ascribable to the necessary storage during transportation. Experiments with one variety, Bramley's Seedling, indicated no influence upon vitamin C value as a result of time of picking, character of soil, age of tree, or season.

TABLE 1
The antiscorbutic potency of apples

VARIETY	LOCATION	MINIMUM PROTECTIVE DOSE, GM
Baldwin	Massachusetts ¹	4
Bramley's seedling	England ²	3
Cox's orange pippin	England ²	20, or slightly more
Dabinett	England ²	10
Delicious	Massachusetts ³	17 to 20
Delicious	Washington ⁴	20 to 25
Golden delicious	Massachusetts ³	12 to 15
Jonathan	Missouri ⁵	20 to 25
King David	Massachusetts ³	13
King Edward	England ²	20, or slightly more
Lane's Prince Albert	England ²	10
McIntosh	Massachusetts ³	More than 20
Newton wonder	England ²	10
Northern spy	Massachusetts ³	4 to 5
Spitzenberg	Massachusetts ³	10 to 11
Winesap	Massachusetts ³	10
Winesap	Washington ⁴	10
Woodbine	England ²	20
Worcester pearmain	England ²	20

¹ Fellers et al., '33.

² Bracewell et al., '30, '31.

³ Fellers (personal communication)

⁴ Present report.

⁵ Hessler and Anderson, '30.

⁶ Potter, '33.

In the United States, Hessler and Craig ('29) found that 20 gm. of Jonathan apples did not protect guinea pigs from scurvy. Hessler and Anderson ('30) subsequently reported that 25 gm. of this variety provided complete protection from September to November, while 40 gm. of stored Jonathans were required from December to May. Fellers ('33) found that more than 20 gm. of McIntosh and 17 to 20

gm. of Delicious were necessary for protection from scurvy. About 13 gm. of King David, 12 to 15 gm. of Golden Delicious, and 10 to 11 gm. of Spitzenberg appeared necessary for protection. Baldwins had a very high vitamin C content, 4 gm. being the minimum protective dose (Fellers et al., '33). The Winesap was found by Potter ('33) to be relatively rich in vitamin C, the minimum protective dose being 10 gm. daily. Potter and Overholser ('33) reported that apples from trees which had received a complete fertilizer (N.P.K.) for 4 years were somewhat richer in vitamin C than were apples from non-fertilized trees of the same district.

The investigation reported herein was conducted with Delicious apples grown in the State of Washington. The vitamin C value was determined in the fall, in the winter, and in the spring. The winter and spring experiments included apples that had been held at two temperatures: about 45°F. ('common storage'), in a fan-ventilated cellar having a relative humidity of 78 per cent to 82 per cent; and 32°F. ('cold storage') in a commercial cold storage plant. The maximum range of temperature in 'common storage' was 38°F. to 48°F.; in cold storage the extreme range was from 30°F. to 34°F.

METHOD

The vitamin C determinations were made according to the method described by Sherman and Smith ('31) except for a few changes here indicated. The duration of the test was in most cases 56 days. The basal diet was modified to contain cod liver oil and yeast because experience in this laboratory as well as that of Eddy ('29) indicates that more uniform results are obtained with a diet so modified. To supplement the iodized salt used and to insure an adequate amount of iodine, distilled water containing about 2.3 parts per million of iodine, added as potassium iodide, was given twice weekly. The amount of apple fed was not adjusted to the weight of each guinea pig as suggested by Sherman. Except in the winter tests, the weights of the animals were approximately 300 gm. in all cases. The animals were weighed every 2 days.

Weighed amounts of the basal diet were given every day and the net intake determined every 4 days.

MATERIALS

Through the courtesy of Dr. E. L. Overholser, of the Division of Horticulture of the Agricultural Experiment Station of the State College of Washington, Delicious apples, extra fancy grade, were obtained from two orchards, one at Chelan Falls and one near Monitor, Washington. No fertilizers had been applied to these trees for 3 years and 2 years respectively. The apples had been washed before packing to remove arsenic and lead residues to below the tolerance of 0.01 grains As_2O_3 and below 0.025 grains of Pb per pound of fruit.

Three sets of determinations were made: a) in the fall from October to December, b) in the winter from January to March, and c) in the spring from March to May. The fall determinations were made on apples held at 32° F. since presumably there would be less destruction of vitamin C at this temperature and the results would, therefore, represent freshly picked apples as nearly as possible. About 11 weeks elapsed between the picking and the final feeding of these apples to the guinea pigs. The winter and spring tests included apples that had been stored at 45° F. as well as those held at 32° F. The apples were washed, wiped, quartered, and cored. Any bruised tissue was removed. Radial sections were fed in which the natural proportions of skin and flesh were retained. Feedings were made 6 days per week.

EXPERIMENTAL RESULTS

The results obtained are summarized in figures 1 and 2. 'Positive controls' on orange juice and on cabbage are also shown.

Prevention of scurvy. The severity of scurvy, as indicated by the scurvy score, varied inversely with the amount of apple fed except that in the fall the guinea pigs receiving 5 gm. of apple showed a slightly higher scurvy score than their negative controls, probably because they lived about 10 days

longer so that the symptoms had more time in which to develop. Of the six receiving 10 gm. of apple, two died and all showed severe scurvy symptoms and a high scurvy score.

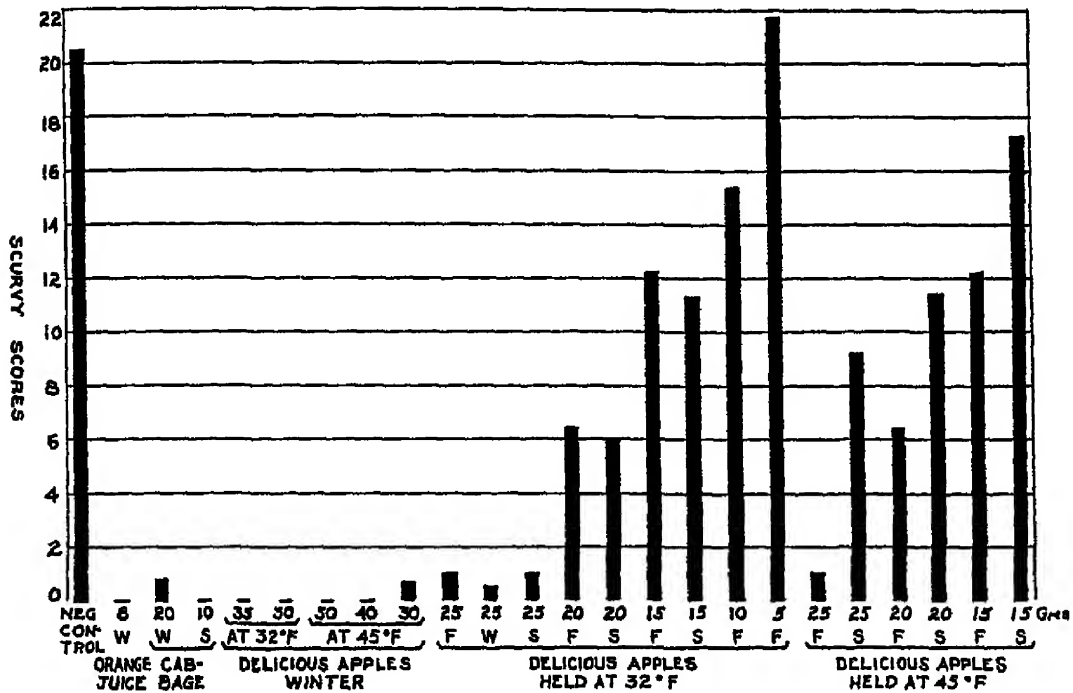


Fig 1 Average scurvy scores of guinea pigs fed a vitamin C-free diet plus supplementary foods as indicated at foot of each column. F = fall, W = winter, S = spring.

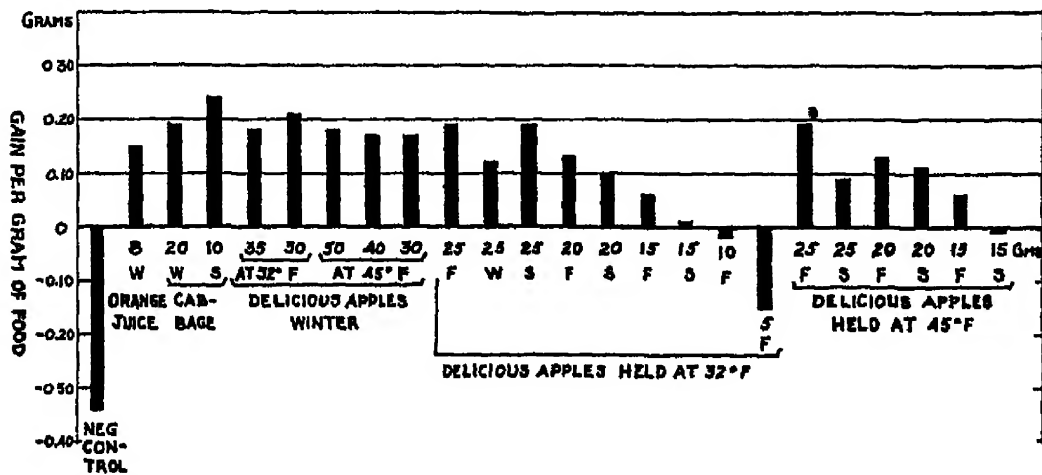


Fig. 2 Average gains per gram of basal diet of guinea pigs fed a vitamin C-free diet plus supplementary foods as indicated at foot of each column F = fall, W = winter, S = spring.

All the animals fed 15 gm. of apple lived but showed severe scurvy. Those fed 20 gm. showed mild scurvy. With 25 gm. complete protection was given at all seasons with apples

stored at 32°F. Experience indicates that such apparent defects as contributed to the very low scurvy score of 1 in the spring and fall are probably of chance origin. No evidence of scurvy was found in the guinea pigs fed 25 gm. of 'cold storage' apples in the winter. A comparison of the results at three levels of feeding (15, 20, and 25 gm.) for the three periods of time shows no diminution in the anti-scorbutic potency of the apples stored at 32°F. for 6 months and indicates that the minimum protective dose lies between 20 and 25 gm. fed 6 days per week.

With the guinea pigs fed the apples stored at 45°F., however, a marked increase in the spring scurvy score is observed for each of the three levels of feeding (15, 20, and 25 gm.). Twenty gm. of 'common storage' apples in the spring resulted in a scurvy score nearly identical with that produced by 15 gm. of apple in the fall. While a direct numerical comparison cannot be made at the other two levels of feeding, a similar relationship is apparent. This represents an increase of one-third in the amount of apple required to provide a similar protection from scurvy which, conversely, may be taken to indicate a loss of about one-fourth of the vitamin C potency of Delicious apples when stored for 6 months at 45°F. The fact that 50, 40, and 30 gm. of apples held at 45°F. gave protection from scurvy in the winter tests indicates that the destruction after 3 months' storage was not more than one-sixth, and possibly less, since no direct comparison was made for the 25-gm. level at that season and temperature.

Gain in weight. The net gain or loss in weight of animals suffering from varying degrees of scurvy has not, in the experience of some investigators (Richardson and Mayfield, '32), shown a direct relationship to the scurvy score. While considerable variability is apparent, particularly in averages of small numbers of animals, the average daily gains of the guinea pigs in these tests (where equal numbers of each sex were compared as far as possible) decreased as the scurvy score increased. It appears, therefore, that in these experi-

ments lack of vitamin C could be correlated directly with the growth failures observed. It is possible, of course, that the vitamin C-free diet lacked some unrecognized growth factor as well as the antiscorbutic factor and that both are present in Delicious apples in approximately equivalent proportions. A study of the efficiency with which the basal diet was utilized for growth seems to indicate, however, that lack of the antiscorbutic factor interfered with the absorption or metabolism of materials essential for growth. The average gains per gram of food ingested per day were similar for all guinea pigs receiving a protective dose of apple or other source of vitamin C but decreased as the allowance fell below the protective level. Apparently, therefore, vitamin C may be held responsible, indirectly at least, for the failure of the scorbutic animals in these experiments to make the gains in weight to be expected from the amount of food they ingested.

GENERAL DISCUSSION

These results indicate that Delicious apples contain about 0.04 to 0.05 unit of vitamin C per gram, or 1.1 to 1.4 units per ounce, according to the standard suggested by Sherman. This represents a relatively low value as compared with some other varieties tested. The outstanding place of Delicious, from the point of view of production in the United States (there were more Delicious trees than of any other variety in 1928 (Hampson, '33)), makes it of more than theoretical interest to consider the dietetic value of this fruit, particularly its value in vitamin C. The Delicious is generally eaten raw, a point worth considering, since, with few exceptions, cooking destroys or drastically reduces the vitamin C content of apples (Bracewell et al., '30; Fellers et al., '33; Kohman et al., '24; Hessler and Williams, '29). Until some standards can be set up which can safely be relied upon to preserve the vitamin C in cooked apples the use of the raw fruit should be encouraged in those households where, for reasons of economy as well as of taste, apples are depended upon to furnish a considerable part of the family's vitamin C require-

ment. Except for infants and young children, who should receive their vitamin C in some such concentrated and readily digested form as tomato juice or orange juice, raw apples might well be used more extensively, especially where they represent a relatively cheap source of vitamin C. The varieties of apple richest in vitamin C would obviously contribute, weight for weight, the largest amount of vitamin C to the diet. But a very large-sized apple like the Delicious, which averaged 200 gm. per apple in these tests, and which is nearly always eaten raw, may contribute an amount of vitamin C to the diet equivalent to that provided by an apple from one of the smaller though richer varieties, particularly those that are frequently cooked before serving. Since the peel contains a greater concentration of vitamin C than the flesh of the apple (Bracewell, '31; Fellers et al., '32), unpared apples provide more vitamin C than those from which the skin has been removed.

Since cold storage preserved the vitamin C content of Delicious apples successfully while 'common storage' permitted some destruction, it appears desirable to use cold storage whenever practicable and to maintain a temperature as near 32°F. as possible, when other types of storage must be used. Incidentally, the apples held at 32°F. retained a better texture and flavor and suffered less from overripeness and other forms of spoilage than those held at 45°F.

SUMMARY AND CONCLUSIONS

Delicious apples grown in Washington were found to have a vitamin C content of 0.04 to 0.05 unit per gram, or 1.1 to 1.4 units per ounce. No loss of vitamin C was evident after 6 months' storage at 32°F. A loss of one-sixth or less occurred during the first 3 months of storage at 45°F., and a loss of about one-fourth during storage for 6 months at this temperature.

Both the growth of the guinea pigs and the efficiency with which they utilized their food decreased as the vitamin C allowance fell below the minimum for protection from scurvy.

It is concluded that Delicious apples, although relatively low in vitamin C as compared to some other apple varieties, may make an important contribution of this vitamin to the diet because they are relatively large in size and are usually eaten raw and unpeeled. It is also concluded that the antiscorbutic factor may have an indirect effect on growth due to failure of the scorbutic animal efficiently to utilize the essential nutrients in the food which it eats.

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STUDIES IN THE CONTROL OF DENTAL CARIES. II ¹

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ONE FIGURE

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Most people who are studying the cause and control of dental caries today believe that a well-balanced diet is a safeguard against the progress of this disease. Various nutritive constituents of the diet are regarded as specific protective agents. Metabolic processes related to calcium or phosphorus utilization or to acid-base balance are thought by many to exert a controlling influence. It is believed that this influence operates through causing changes either in tooth structure or in salivary composition, or by affecting the ability of aciduric organisms to grow in the mouth.

Many of the studies with children that have been reported cover rather short periods of time. Findings on first examination of teeth have had to be taken as describing the degree of activity of caries at the time of beginning the experiment. In some instances conclusions have been based on combinations of clinical and chemical evidence; in others, on bacteriological evidence of a qualitative nature considered in connection with clinical findings. In many instances conclusions have been based on clinical results obtained 6 or 8 months after some change had been made in the diet for one

¹ This work is a part of a group study of the cause of dental caries, being conducted under the auspices of the Children's Fund of Michigan, at the School of Dentistry, University of Michigan.

group of children, in contrast with similar evidence in another group in which the diet had not been changed. Sometimes changes in diet have involved a single dietary factor. In other instances, as in the daily addition of 1 pint of orange juice to the routine diet, many nutritive changes have been introduced.

In our studies we were fortunate in being able to conduct some investigations on children living in an institution. Yearly dental examinations over a period of years had revealed a surprisingly low incidence of dental caries among the children, without any conscious effort on the part of the managers of the orphanage to produce this result. Many of the children have lived in this institution for long periods of time. The same persons have been in charge for 6 or 7 years, during all of which time the same general policies have been followed in meal planning and food service. Due to the interest and cooperation on the part of those in charge, we had an unusual opportunity to study these policies. We hoped that we would be able to find out what dietary or nutritive principles were responsible for the unusual clinical results. At the same time we were able to make bacteriological and some chemical studies on the saliva of a selected group of the children. Distance from the laboratory made it impossible to make more extensive studies in these fields.

The cottage system is used in housing the children. The cottages are attractive and very well kept up. An unusually well-equipped recreation building and play field provide excellent recreational facilities. A large out-door swimming pool is in daily use whenever the weather permits. The children are a well-trained happy group, free from worry and from conditions leading to high nervous tension. The general atmosphere in the institution is that of a well-managed home. Bed time and mid-day rest periods are routine and adjusted to the age and physical condition of the children. Work and play are judiciously intermingled. The younger children attend school on the grounds, while those of junior and senior high school age attend school in the near-by town. The children are not permitted to go away from the institution

except during the summer months. Many of them have no homes to which to go.

Their general health has been very good. There is a hospital on the grounds with two nurses in constant attendance. With such facilities infectious diseases do not have as much chance of spreading as they do among children living in private homes and attending public schools. A pediatrician and a dentist from the near-by city are responsible for the necessary medical and dental care.

METHODS

The children are weighed and measured in May and November each year. These records were available to us as were their medical records. Inasmuch as the same policies in planning meals had been followed for several years previous to November 1932; the records at the end of May 1932 were used as the basis for the data presented in figure 1. This chart shows graphically the proportion of boys and girls of different age groups that varied from the generally accepted normal range of height and weight. The extent of individual variation is also indicated. The younger children had lived under this institution regime for an average of $2\frac{1}{2}$ to 3 years, the older ones for an average of 5 or 6 years or more. Woodbury tables were used in calculation of so-called normal weights for age and height and of normal heights for age.

After the children are weighed in November, it has been customary each year, to give one teaspoonful of cod liver oil once a day to those children who are conspicuously under weight for their age and height. This dosage is continued through the winter months only. No special attention has been given, however, to those markedly under average height for their age.

Dental examinations. In October 1928, our group first began to make yearly examinations of the teeth of the children living in this institution. Those who were over 6 years old at that time were selected. It has not been possible to have

bite-wing x-rays taken, but individual charts have been kept. Successive findings from year to year have been recorded on these charts with inks of different colors. Each succeeding year until October 1931, examinations were limited to this same group of children. By October 1931, this original group had been considerably reduced in numbers, due to withdrawals

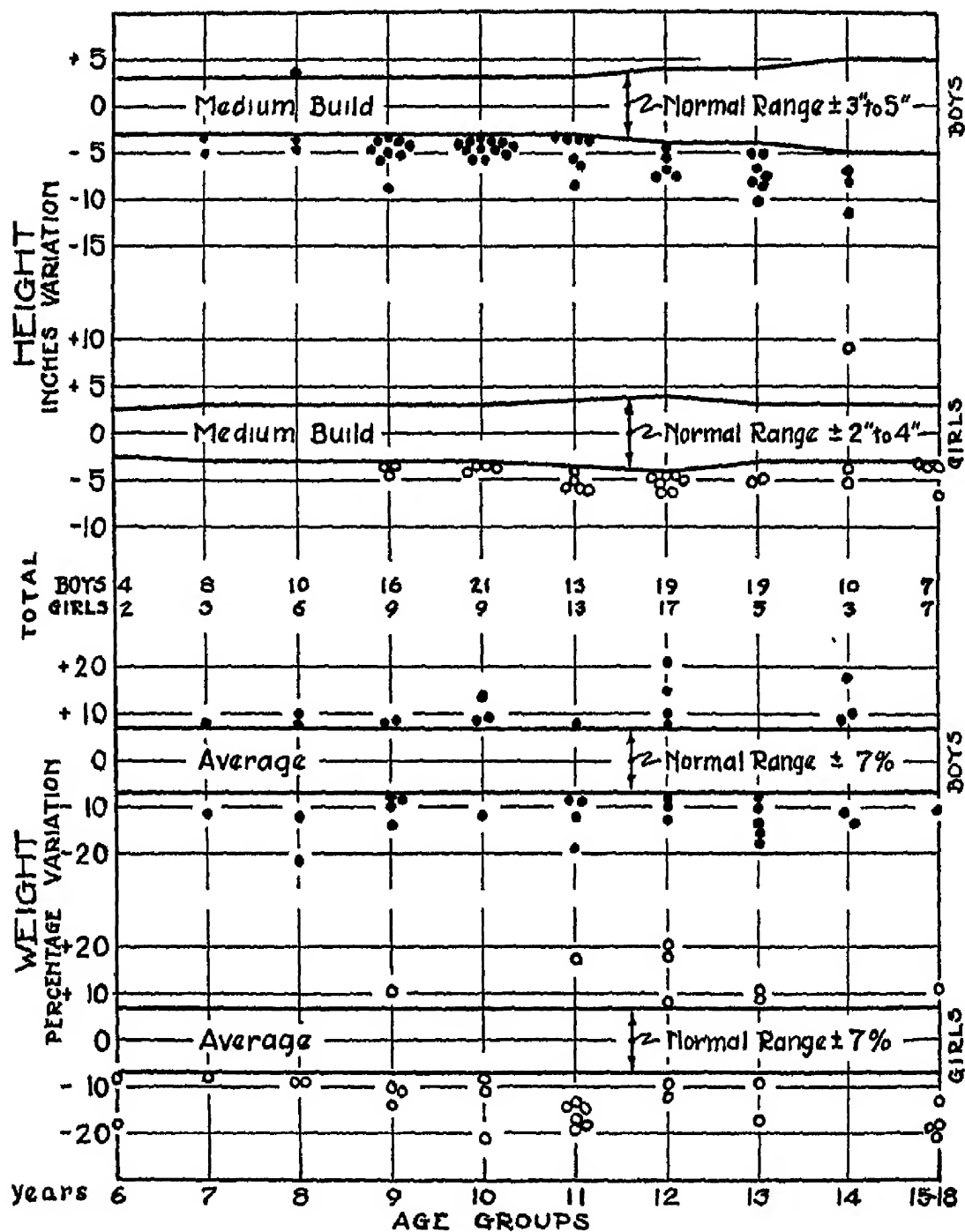


Fig. 1 Abnormalities in height and weight in a group of 127 boys and 74 girls who had lived in an orphanage for an average of 4 years.

from the institution. All other children over 6 years old were then added to the list. From October 1928 to October 1931 only very urgent dental conditions in the children who were under our observation were cared for by the local dentist. Most cavities were left open.

The last examination was made in June 1933 instead of October, because of the probable necessity of our researches having to be discontinued. At the end of June 1933 we were in possession of yearly dental records for 49 children covering $4\frac{1}{2}$ years, for 109 others $1\frac{1}{2}$ years and for 11 covering 1 year. At this time all individual records were carefully studied and a yearly diagnosis made for each child describing the progress of dental caries during each year. The following symbols were used:

C—indicated that no new cavities had developed. If used for the description of the result of the first examination, it meant that the child had had no cavities at that time.

C± was used to represent a very slight amount of activity.

C+ signified a fair amount of activity.

C++ represented considerable activity.

C+++ indicated extreme susceptibility.

In each case the symbol represented change since the last previous examination. In table 2 will be found a summary of the progress of caries in these children, classified according to the periods of time indicated.

Diet. During the year 1931 our efforts were directed to general observations on the food the children were receiving and to securing full information from the person in charge, concerning the general policies that had guided her in planning their menus. We have copies of the menus for several years back. Comparison of these with the statements made gave a close check on the accuracy of her description of these policies.

Children past 8 years old received milk and oleo routinely for breakfast. Occasionally these foods were served for supper. Butter was not available. A high proportion of bread and other cereal foods was used regularly. Many

vegetables and some fruits are raised each year on the institution farm. Apples, carrots, tomatoes, onions, potatoes, cabbage, beets and turnips were abundant at all times. Celery, spinach, peas, string beans, corn, berries, etc., were given to the children in season. Carrots, onions, cabbage, turnips and tomatoes were frequently served raw. Oranges were given to the older children only during the Christmas holidays, except as sent in to individual children at other times by relatives. Canned berries, peaches and apples, together with dried prunes and peaches were purchased for their menus; fresh bananas were purchased occasionally. Eggs were available to the children in the spring, during which period they were produced on the farm in considerable quantity. The only desserts that were given regularly to the children were fruit gelatin² for Sunday dinner and cookies that were served to them once a week. Raw apple was used in place of dessert for dinner and supper at practically all other times. Fresh and smoked meats were purchased for the children's menus. Meat was served almost every day, but portions were quite small. Candy that was sent in to the children was not given to them. Parents were urged to limit food gifts to fresh fruits.

There were three features about this general type of diet that seemed to us might bear a relation to the low incidence of caries among the children. One was the habitual use of hard fibrous raw fruit at the close of meals. Another was the fact that foods containing added sugar were rarely used. Lastly, the food was always of uniform quality and was eaten at regular times.

Because all of the children ate in the same dining room, it was thought unwise to attempt to arrange for groups of children to receive different dietary supplements of interest to us in our research program. A group of 60 girls, all living in the same cottage and all over 6 years of age, was selected, however, early in January 1932. We wished to observe whether or not the order in which raw fruit and vegetable

² Jello.

was eaten at meal time made any difference in dental or bacteriological findings. For 1 year these children ate their raw fruit at the beginning of meals instead of the close as had been the usual custom. All other children continued their customary practice in this respect. Twenty girls of different ages from this special group and an equal number of similar ages from among the rest of the children were selected as subjects for making bacteriological studies on the saliva.

Eight each from these 2 groups of 20 were selected to study the food intake of representative children in the dining room. Three such dietary studies were made. Because of the general uniformity in types of meals served, we were certain that the children received the same kind of food while our studies were in progress as they would have received otherwise. On our part we followed the same policies in food service to the diet group that were followed throughout the dining room. Before each meal we obtained information from attendants in the dining room as to whether second helpings were to be given, size of first helpings, etc. Food was prepared separately for the 16 whose food intake was being studied, but proportions were kept similar to those used in preparing the larger quantities. The cooks weighed all constituents of various foods served to the diet group and weighed the final cooked product for us. All food consumed by these 16 girls during the time dietary studies were in progress was weighed.

The 3 studies covered the following periods: 3 days at the end of May 1932, 4 days in September 1932 and 7 days in March 1933. As far as possible the same girls were studied each time. Because of a few withdrawals, however, there had to be occasional substitutions. New children were always selected from the 2 groups of 20 to which we have already referred. The results of May and September 1932 studies were combined, making a full week for comparison with the week studied in 1933. As a basis for our calculations we used the compilation of generally accepted data on food composition that has been published by the Department of Dietetics, University Hospital, Ann Arbor (Waller, '32). Table 1 gives

a summary of these results together with a summary of similar data covering 54 days, for 9 girls who were in our special diet group at University Hospital (Koehne et al., '34; Koehne and Morrell, '34).

In November 1932, after our dietary studies for the two periods in 1932 had been completed, we secured the cooperation of the physician and of those in charge at the institution in making a few simple changes in policy. These changes resulted in the children thereafter receiving regularly more milk and egg, some butter and a greater proportion of whole cereal products than of the milled varieties. The hot dishes served for dinner and supper were higher in nutritive value and stewed fruit was included in the breakfast menu. Further changes were not deemed wise in view of the stress of general financial conditions. Table 1 shows a direct comparison of food intake, as measured in March 1933, 4 months after these changes were instituted, with the results previously obtained.

Bacteriological studies. From December 1931 through April 1933 quantitative estimations of the concentration of *Bacillus acidophilus* in saliva were made once a month for the selected group of 40 children previously described. The method developed by Hadley ('33) was used in this work. No tests were made during the summer months. Thirteen such determinations were made for 25 of the original group. There were 15 withdrawals during the first year. Qualitative tests for the presence or absence of aciduric organisms in the saliva were made at each time that quantitative determinations were made. The method used was that which has been described by Jay and Voorhees ('27) and by Bunting, Nickerson and Hard ('26).

Chemical studies. During the latter part of May 1932, and in March 1933, while studies of the food intake of 16 girls were in progress, the carbon dioxide capacity of the saliva of the same girls was measured. The macro method used was the one developed by Van Slyke and Cullen ('17) for similar determinations on blood serum. One cubic centimeter of saliva was used for each determination. The saliva was col-

*Comparative study of the food intake of sixteen girls living in an orphanage
and of nine girls at University Hospital*

	ORPHANAGE		HOSPITAL
	1 week 1932	1 week 1933	First 6 to 11 weeks in hospital
Average age (years)	10.9	12.0	8.87
Years spent in institution	3.7	4.3	
Average height (inches)	52.5	55.37	52.37
Deviation from normal height/age	- 3.2	- 2.54	+ 0.8
Normal range of deviation	+ 3.2	+ 3.4	+ 3.0
Average weight (kilograms)	29.6	35.3	30.8
Deviation from normal weight/height/age	- 4.0%	- 0.3%	+ 3.0%
Normal range of deviation	± 7.0%	± 7.0%	± 7.0%
Protein: average daily grams	61.8	75.8	74.0
Grams per kilogram	2.1	2.25	2.4
Per cent from animal sources	40.7	43.1	65.75
Fat: average daily grams	59.6	74.8	99.0
CHO: average daily grams	249.0	299.0	233.0
Added sugar: average daily grams	15.0	15.3	29.2
Calories: average daily	1759	2169	2074
Calories per kilogram	60.8	64.2	66.4
Calories per inch height	33.8	39.3	39.5
Per cent from protein	13.9	14.0	14.2
Per cent from fat	30.3	31.0	42.8
Per cent from CHO	56.0	55.1	43.0
Per cent from added sugar	3.4	2.8	5.6
Calcium: average daily grams	0.64	0.99	1.39
Phosphorus: average daily grams	1.11	1.52	1.54
Iron: average daily milligrams	12.0	13.7	13.9
Acid value: cubic centimeter N/1 acid	20.9	23.4	19.0
Base value	32.8	43.8	49.4
Excess base value:	11.9	20.4	30.4
Average amounts of different kinds of food eaten daily (grams)			
White bread	131	84	0
Whole wheat bread	54	91	71
Other cereal foods	261	152	96
Cooked dry legumes	51	61	0
Totals	497	388	167
Milk, all purposes	397	632	985
Potatoes	93	96	141
Raw vegetables, fruit, tomato juice	197	153	189
Cooked vegetables and fruit	138	226	251
Totals	428	475	581
Butter	0	12	29.5
Oleo	11	7	0
Miscellaneous: fat-rich foods	19	13	33
Cane sugar	2	2	4
Miscellaneous sweets	12	7	10
Eggs	8	21	38
Meat, fish, poultry	49	21	66
Nuts, peanut butter	8	12	2
Cheese	0	16	9
Totals	65	70	115
Percentage distribution of calories among different classes of foods			
	¹		
Cereals, bread, legumes	44.3	39.6	15.8
Milk	16.3	20.3	31.8
Vegetables and fruit	12.9	14.9	17.1
Fat-rich foods	10.1	13.3	18.1
Sweets	4.7	3.2	5.7
Meat, egg, cheese, nuts	11.7	11.3	11.4

¹ Daily caloric intake markedly low during this period. Results do not have the significance they should have, if caloric intake had been adequate as in other two studies.

lected under oil, through a long-stemmed glass funnel. Saliva flow was always stimulated by the slow chewing of a small piece of paraffin.

DISCUSSION

Diet in relation to the height and weight of the children. The results of the dietary studies made during 1932 are reported in table 1. As evidence that this summary for 16 children is representative of the food intake of the children who lived in the institution during the preceding 6 or 7 years, we submit the data found in figure 1. Only 1 boy and 1 girl were taller than the average tall child of their ages. Forty-nine of the 127 boys represented were shorter for their age than the average boy of short stature. The same was true of 27 of the 74 girls. As will be seen by reference to figure 1 there was a massing of this condition from 9 years of age on for both sexes.

Weight data presented in figure 1 should be interpreted in comparison with the height record. Estimation of the so-called normal weight was made, as is customary, according to the age and actual height of each child, not according to his so-called normal height for age. Twenty-two of the boys were more than 7 per cent below normal average weight for their age and height, while 16 were more than 7 per cent above this average value. In the case of the girls 26 were below this normal range in weight for their age and height and 8 were above this average range. Figure 1 indicates that the distribution between over and under weight was more uniform in the boys of the different age groups than among the girls. In most age groups a higher percentage of the girls were under than were above the average weight range for their height and age. Within a period of 6 months following November 1932 when the changes were made in diet, there was a doubling of the gain in weight and a 15 to 20 per cent increased gain in height in a group of 85 boys and 54 girls, in comparison with gains made by those same children in a similar 6-month period the year before.

Relation of dental findings to height-weight status, age and sex. A study was made of the possible correlation between the height or weight status of the children and their degree of susceptibility to caries. About the same proportion of children who were stunted in height and were more than 7 per cent above or below average in weight for their height and age, was found in each of the dental classifications.

There are 109 boys and 60 girls represented in table 2. The group in whom caries remained inactive for the periods of time under observation included 55 boys and 28 girls. Fifty-

TABLE 2

Summary of dental findings for 169 children who were under observation for varying periods of time

PERIOD UNDER OBSERVATION	4½ YEARS		1½ YEARS		1 YEAR	
	NUMBER OF CHILDREN		NUMBER OF CHILDREN		NUMBER OF CHILDREN	
	88 boys	16 girls	68 boys	41 girls	9 boys	2 girls
	Number	Per cent	Number	Per cent	Number	Per cent
Immunes	6	12.2	26	23.8		
Inactive since first examination	17	34.7	52	47.7	9	81.8
S1 to moderate activity for first 1 to 2 years, inactive for last 2½ to 3½ years	5	10.2				
S1 activity throughout period	9	18.2	20	18.3		
Moderate activity throughout period	8	16.3	8	7.3	2	18.2
Considerable activity throughout period	4	8.1	3	2.7		

five per cent of these boys were under 10 years of age in contrast with 40 per cent of the girls. Eighteen boys and 7 girls exhibited moderate to very active caries while under observation, 7 of the boys and 4 of the girls being under 10 years of age. Two-thirds of the 19 boys and 13 girls classed as immunes were under 10 years old. It seems significant that much the larger proportion of so-called immune children was found in the younger age ranges. Likewise almost twice as high a proportion was found among those under observation for just 1½ years as was found among the 49 who were observed for 4½ years. Time seems to lower considerably the

proportion of individuals who can be called immunes. We hope to continue our observations on these children in order to determine how many of the younger ones remain immune over a period of years.

Relation between dental and bacteriological findings. Ten to 13 tests were made on the salivas of each of 25 girls between December 1931 and April 1933, to determine the concentration of *Bacillus acidophilus* in the saliva. Twelve of these girls belonged to the special group who, throughout the year 1932, ate their raw fruit and vegetable at the beginning of meals. The remaining 13 were of similar ages but had always eaten their raw fruit and vegetable at or near the close of meals. None of the 25 girls belonged to the group of immunes, for all had had cavities on first examinations.

In table 3 will be found the dental diagnoses and the bacteriological findings, qualitative and quantitative, for 9 representative children in the group of 25. Wherever the quantitative plate is recorded as negative but the qualitative test as positive, it may be assumed that there were probably less than 100 aciduric organisms present per cubic centimeter of saliva. For purposes of routine interpretation in this laboratory, it has been customary to consider counts less than 5000 per cubic centimeter as low, those between 5000 and 50,000 as moderate, and those over 50,000 as high. The continuous presence of these organisms in the saliva over long periods of time is typical of caries activity. In actively susceptible cases, the counts usually vary from moderate to high, although some may be low.

Two additional children had records resembling that of V.S. Such records are typical of caries inactivity, yet the November 1932 dental examination for V.S. and D.B. showed slight activity as did the June 1933 examination for A.R.P. These 3 cases and 1 other (R.B.), who had a bacteriological record resembling that of J.G. and a dental record similar to that of V.S., were exceptional cases in that their records failed to show a direct correlation between the dental and the bacteriological findings.

The salivas of 3 children were consistently negative to *B. acidophilus*, both qualitatively and quantitatively, and none developed cavities during the year and a half of observation. One other child had a record resembling that of A.K., while another child's record was similar to that of L.P. Eight other children had records like that of J.G.

TABLE 3
Dental and bacteriological findings for nine representative children

DATE		V.S	J.G	A.K.	L.P.	W.M	B.STA	V.U	B.STE	R.S
Oct 1931	Diagnosis	C+	C+	C+	C+	C++	C+	C+	C+	C++
12-18-31	Quant. test	0	0	0	0	0	0	36,075	0	4,475
	Qual. test	neg.	neg.	neg.	neg.	neg.	neg.	pos	pos.	pos.
1-4-32	Quant. test	0	0	400	370,000	0	0	75,000	37,312	8,375
	Qual. test	neg.	pos.	pos.	pos.	neg.	neg.	pos.	pos.	pos.
1-21-32	Quant. test		0		0	0	0	9,075	1,200	0
	Qual. test		neg.		pos.	neg.	neg.	pos.	pos.	pos.
3-3-32	Quant. test	0	0		0	0	0	10,775	8,075	43,500
	Qual. test	pos.	neg.		neg.	neg.	neg.	pos.	pos.	pos.
4-5-32	Quant. test	0	0	0	0	0	0	8,700	216,000	0
	Qual. test	neg.	neg.	neg.	neg.	neg	neg	pos.	pos.	pos.
5-9-32	Quant. test	0	0	0	0		0	0	27,000	
	Qual. test	neg.	neg.	neg.	pos.		neg.	neg.	pos.	pos
6-6-32	Quant. test	0	0	0	0		0	0	288,000	0
	Qual. test	neg.	neg.	pos.	neg.		neg.	neg.	pos.	pos.
9-20-32	Quant. test	0	0	0	0	0	1680	0	9,000	0
	Qual. test	neg.	neg.	neg.	neg.	pos.	pos.	neg	pos.	pos
11-1-32	Diagnosis	C±	C—	C—	C—	C—	C—	C—	C—	C+
	Quant. test	0	0	0	0	0	0	0	12,000	0
	Qual. test	neg.	neg.	neg.	neg.	neg.	pos.	neg	pos.	neg.
12-17-32	Quant. test	0	0	0	0	0	302	0	9,075	0
	Qual. test	neg.	neg.	neg.	neg.	neg.	pos.	neg	pos	neg.
1-28-33	Quant. test	0	0	0	0	11,700		0	100	0
	Qual. test	neg.	neg.	neg.	neg.	pos.	pos.	neg.	pos.	neg.
3-20-33	Quant. test	0		0	0	121,600	125	0	8,200	15,062
	Qual. test	neg.	neg.	neg.	pos.	pos	pos	neg.	pos.	pos.
4-16-33	Quant. test	0	0	0	0	22,800	0	0	8,600	11,900
	Qual. test	neg.	neg.	neg.	neg	pos.	pos.	neg.	pos.	pos
June 1933	Diagnosis	C—	C—	C—	C—	C±	C±	C—	C+	C+

Quant. test gives the number of *B. acidophilus* per cubic centimeter of saliva.

The records of W.M. and B.Sta. are typical of caries inactivity changing to activity. The opposite condition is seen in the findings reported for V.U. B.Ste and R.S. have records that are typical of caries activity. In these last two cases we see caries activity associated with rather low counts in R.S. as well as with moderate to high counts (B.Ste.). Undoubtedly variations exist in individuals in rate of development of cavities. Then, too, improvement in methods of collecting saliva for quantitative counts may later lead to results that are more comparable from person to person than are present methods.

Out of the 25 cases discussed, all but 4 show a direct correlation between dental and bacteriological findings, making a correlation of 84 per cent. This compares favorably with the 88.9 per cent correlation found in our hospital studies (Koehne, Bunting and Morrell, '34).

During the year 1932, the incidence of new caries was practically the same among all the children as during the previous years, in spite of the fact that all existing cavities had been filled and all of the teeth cleaned at the beginning of the year. This had not been done in previous years.

Relation between dental findings, diet and carbon dioxide capacity of saliva. The results of the dietary study made in March 1933, 4 months after certain policies in meal planning had been changed, showed approximately a 10 per cent increase in intake of available acid. The excess base intake was about 70 per cent higher than on the type of food consumed previous to November 1932.

Determinations of the carbon dioxide capacity of the saliva of the 16 children in the diet groups of May 1932 and of March 1933 gave the following results:

In May 1932, the average for the group was 20.35 cc. CO₂ per 100 cc. of saliva (range, 11.0 to 31.1). The average of the values obtained in March 1933 was 22.04 cc. CO₂ per 100 cc. of saliva, the values ranging from 10.8 to 30.4. The alkaline reserve of the saliva in March 1933 was only about 8 per cent higher than in May 1932, while the diet showed a 70 per cent increase in available base.

Twelve of the 16 girls in the group in March 1933 had been in the group in May 1932. Seven of these 12 girls had remained inactive to caries since October 1931. In May 1932 the average CO₂ capacity of their salivas was 20.9 cc. per 100 cc. of saliva (range 13.9 to 31.1), while in 1933 it was 22.0 cc. CO₂ per 100 cc. of saliva (range, 15.4 to 30.4).

Four of the 12 showed a slight degree of caries activity during the year and a half. In 1932 the average carbon dioxide carrying power of their saliva was 21.75 cc. CO₂ per 100 cc. of saliva (range, 13.5 to 25.0); in 1933 it was 23.6 (range, 17.6 to 28.5).

The one child who was definitely susceptible to caries had a CO₂ capacity for the saliva of 11.0 cc. per 100 cc. of saliva in 1932 and of 13.3 in 1933. These values would have more significance if there had been more children in this group giving correspondingly low values. As it stands the results for this 1 child have to be compared with values of 13.5 and of 17.6 cc. CO₂ per 100 cc. of saliva for 1 child and of 13.0 and of 15.4 cc. CO₂ per 100 cc. of saliva for another child, both belonging in the group that had remained inactive to caries.

As was found in the more detailed studies on a group of children in University Hospital (Koehne et al., '34) whose food intake was accurately measured over rather long periods of time, we were unable to find any significant quantitative correlation between the available base value of the diet and the alkaline reserve of the saliva as measured by its carbon dioxide carrying power. We also found no consistent relationship between the concentration of this component of the saliva and the progress of caries susceptibility. Such a possible relationship had been indicated by Hubbell ('33) in tests made on the saliva of a group of school children living in Ann Arbor.

Dental findings in relation to diet. Attention is again called to table 2 which presents a summary of the progress of caries among the children living in this institution. It will be regarded as unusual for 57.1 per cent of a group of children of

these ages to remain immune or inactive to the progress of this disease for the last $2\frac{1}{2}$ to $4\frac{1}{2}$ years that they were under observation. This proportion was increased to 71.5 per cent when observations were limited to $1\frac{1}{2}$ years, and to 81.8 per cent for those observed for only 1 year. Only those children are listed as immunes who were free of caries on admission and who remained free of cavities during the time indicated. The usual amount of decay was found among all other children on first examination.

No higher incidence of caries was noted in the group of 60 girls during the year in which they ate their raw fruit and vegetable at the beginning of meals than had been found in the same children during previous or subsequent periods or than was observed among the other children who had continued their customary practice of eating such food at or near the close of meals. This observation was confirmed in carefully controlled studies made on some of the children in our diet group at University Hospital (Koehne et al., '34). Also, in these latter studies, we found that no special advantage was gained in controlling caries by eliminating the few simple desserts in our standard diet (table 1, right hand column) and substituting raw apple.

Between the October 1932 examination and that of June 1933, during which time the nutritive value of the diet showed considerable improvement, there was no apparent change in degree of susceptibility over that noted in previous years. With the exception of this final 6 months' period, the type of diet had, for several years, corresponded to that summarized in the left hand column of table 1. Therefore the low incidence of caries noted in table 2 had prevailed on a diet in which 40 to 45 per cent of the caloric intake was from starchy food, largely refined cereals.

Mellanby and Pattison ('32) have claimed that high cereal consumption encourages decay of the teeth by interfering with calcium and phosphorus utilization, through increasing the need for vitamin D. Hawkins ('31) has insisted that high cereal consumption favors the progress of caries by encourag-

ing film formation because of the gluten content of wheat. If either of these claims are justified, why has not caries been rampant in this institution? The children's mouths have remained unusually clean in appearance. Calcium and phosphorus intakes, except for the last 6 months, were consistently lower than usually advocated for growing children. The children play out of doors a great deal, but probably no more than the majority of children attending public schools and living at home. The small doses of cod liver oil given, for several months each winter, to children who were considerably under weight, could not account for the low incidence of caries in the whole group. We found approximately the same incidence of caries among the underweight children who had been given this dosage of cod liver oil for the past 1 or 2 winters, as indicated in table 2 for the whole group. We therefore do not feel justified in concluding that vitamin D was responsible for the clinical findings. Certainly the low incidence of caries cannot be explained on the basis of optimum intake of calcium and phosphorus.

We studied the incidence of caries separately among the children who had attended junior or senior high school for the past 1 or 2 years, compared with that found among those attending school on the grounds. Those attending junior high school return to the institution for lunch; those attending senior high school carry their lunch to school. Both of these groups of children assist in the children's dining room or in the other dining rooms on the grounds, employees', nursery, hospital, etc., and have some access to foods not available to the younger children. Again, however, the rate of incidence of caries was approximately the same, as among the whole group.

It has seemed to us that there are two possible dietary explanations for the clinical findings. One is the extremely low intake of artificially sweetened food. The other is the absolute regularity of meals and the uniformity in the quality of food served. Perhaps there are other explanations not evident to us at this time.

Drain and Boyd ('30), Hawkins ('31), Davis ('29) and others have publicly recommended the use of types of diets which have given satisfactory results in arresting the progress of caries in a high percentage of their cases. All have recommended well-balanced diets with restriction in the use of highly sweetened food. Hawkins has also insisted upon a low intake of cereal food. Davis has prescribed, in addition to the diet, the use of certain of his patent preparations. Each has explained the resultant beneficial effects according to his own theory. We can find no published evidence that diets, which include the unrestricted use of artificially sweetened food, have given successful results in stopping the progress of caries in large numbers of cases. Many groups of primitive people can be found who are free of caries. Their diets are usually limited to a few simple natural foods.

Results of experiments conducted by us on a group of children in University Hospital (Koehne et al., '34) living under strictly controlled conditions, have seemed to indicate that a well-balanced diet containing restricted amounts of sugar, is able to control the progress of caries in a rather high percentage of cases. Diets equally valuable nutritionally but containing a high percentage of calories from sweets, seemed to facilitate its progress. In these orphanage studies, however, we found that equally good results were obtained on a larger number of children observed for longer periods of time, when the children were on a type of diet much less satisfactory nutritionally but even lower in sugar content than that used by us in our hospital group. (Compare data given in table 1 for details of diets used by the two groups of children.)

Observations made by us on a group of children attending public schools in Ann Arbor and living in their own homes have seemed to indicate that irregularities in meals and in type of food eaten day by day, associated in most cases with the liberal use of artificially sweetened food, encouraged cavity formation.

If, during the past 6 or 7 years, the children in this institution had received a type of diet similar to that indicated in the right hand column of table 1, we are unable to say whether or not the incidence of caries would have been still further reduced. This type of diet corresponds to that recommended by Boyd and Drain and others. We are confident that the use of the better balanced diet would have eliminated most of the abnormalities in height and weight recorded in figure 1.

It will be noted on referring to table 1 that, on the regime preceding November 1932, the average daily caloric intake was below that deemed desirable for growing children of these ages. Suppose, as is done in many orphanages, that this institution had made a practice of accepting all gifts of confectionery which are so frequently made by well-meaning business firms, and had dispensed such food freely to the children. Undoubtedly there would not have been a deficiency in caloric intake. Since the children have excellent appetites, they probably would have continued to eat everything available in the dining room. The general diet would have been just the same, the caloric intake would have been adequate instead of deficient, but a much higher proportion of the calories would have been derived from added sugar. What would have been the effect of such a regime on caries incidence? We offer no prediction, but we know several similar institutions that follow this practice. The yearly incidence of caries among their children corresponds more to that found among public school children.

The secret of controlling caries activity through diet may, for the majority of persons, lie more in what is withheld from the diet than on the nutritional adequacy of what is permitted. The results of our observations in this institution would seem to point in this direction.

In conclusion we wish to call attention to one factor, the importance of which has been brought home to us many times in our recent studies and which may explain some of the discrepancies in published data. It is not safe, in dealing with human subjects, to base conclusions as to the effectiveness of

various experimental measures in arresting caries solely on short time observations of clinical results. The incidence of caries in the same group in a similar period preceding the experimental periods, should be reported for comparison. Cavities may remain unchanged for long periods of time.

Only 32 of the 169 children represented in table 2 were free of caries when examined in October 1931. The remaining 137 had teeth showing varying amounts of decay, open cavities in many instances. If we had begun to give viosterol or other specific treatment in October 1931, without any previous knowledge of caries incidence among the children, and had based our conclusions on clinical findings in October 1932, we would have been able to present striking evidence for the beneficial effect of our treatment, whatever it had been. Yet 22 of these 137 children had had no new cavity formation for 1 to 3 years previous to October 1931. During the following year, which was the first year of observation for all but 49 of the children, a total of 83 showed no progress of caries activity, even though no fundamental change had been made in the dietary or in other conditions.

SUMMARY AND RESULTS

1. The progress of caries is reported among 169 children living in an orphanage. They were under observation for periods varying from 1 to 4½ years.

2. Improvements made in the diet of the children brought about marked improvement in the height and weight of most of them and some increase in the alkaline reserve of the saliva. This latter increase was not at all proportional, however, to the increased alkaline value of the diet.

3. Bacteriological tests on the saliva of a selected group of these children gave results that checked with the dental findings in 84 per cent of the cases studied. The small percentage of cases in which the dental findings could not be satisfactorily explained on the basis of the quantitative bacteriological findings was slightly lower than observed in our studies of children in University Hospital.

4. No correlation could be found between the dental findings and any of the following factors:

- a. Height or weight status of the children.
- b. Sex.
- c. The carbon dioxide carrying power of the saliva.
- d. The intake of calcium or phosphorus or vitamin D.
- e. Probable access to other food.
- f. The order in which hard fibrous fruit was eaten.

5. The low incidence of caries among the children could not be explained on the basis of the superior nutritional value of their diet. Almost half of the caloric intake was from starchy food.

6. There were two other dietary factors that may explain why the incidence of caries was so low in this institution. One was the low intake of artificially sweetened food. The other was the great regularity of the meals and the uniformity in quality of the food served. If these were the controlling factors, we offer no explanation of how they operated. We merely submit our evidence in support of them.

Acknowledgment is made of the assistance rendered by Maxine Turner and Adelia Grieber, department of dietetics, University Hospital, for assistance in making some of the dietary studies. We wish to express to the superintendent and to his wife, who was in charge of food, our appreciation for their constant interest and careful cooperation in every detail of these studies.

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URINARY EXCRETION OF CITRIC ACID

I. EFFECT OF INGESTION OF LARGE AMOUNTS OF ORANGE JUICE AND GRAPE JUICE

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Increase in the alkalinity of the urine and in organic acid excretion resulting from the ingestion of fruits or fruit juices has been reported by Blatherwick and Long ('22), McLaughlin and Blunt ('23), Chaney and Blunt ('25), Saywell ('32), Saywell and Lane ('33), and Clouse ('33). In general this increase in organic acid excretion when fruit juices are fed has been interpreted to mean that the ability of the body to oxidize the particular organic acids represented has been overstepped, with the consequent elimination of the excess in the urine. There is some evidence, however, that the organic acids eliminated in the urine are not remnants of unoxidized fruit acids, but that they are of metabolic origin. In this connection, Clouse cites the increase in uric acid excretion obtained by McLaughlin and Blunt ('23) on a diet high in fruits. Clouse ('33) found that the ingestion of large amounts of orange juice and grape juice likewise increase uric acid excretion.

Recently the functioning of organic acids as a part of the acid-base balance regulatory mechanism of the body has received considerable attention. Increase in the total organic acid output of the urine on administration of alkali in the form of sodium bicarbonate was noted by Fanconi ('28) and by Fasold ('30), which suggests that organic acid radicals of

metabolic origin furnish a means of freeing the body of excess base. The increased alkalinity produced by fruits and fruit juices might then account for the increased organic acid excretion observed when these are fed in large amounts.

Of the organic acids concerned in acid base balance regulation citric acid has received the most attention and is the one which at present is being most extensively investigated. Its occurrence in the urine was first demonstrated by Amberg and McClure ('17) who considered a direct food origin very improbable. Fasold ('30) concluded from one of his studies that a considerable part of the citric acid excreted is of food origin, but, at the same time, he suggests a connection of citric acid with an acid-base balance regulatory mechanism. In another study ('30) he presents additional evidence for the latter view, which is further supported by the work of Östberg ('31).

It then appears that the assumption that the excreted organic acids can be taken as a measure of the unutilized ingested organic acids is open to question. It was felt that this subject might profitably be further investigated by following a particular organic acid contained in fruit juices, determining in every case both the amount of the acid ingested and the amount excreted. In none of the fruit juice studies reported has this been done. Citric acid was chosen as the particular acid of investigation because of the interest in it as a component of an important class of fruits and as a probable metabolic product functioning in acid-base balance regulation. Orange juice and grape juice were selected because of the readily available data from their use in other studies on urinary acidity. Their great difference in citric acid content also commended them for the purpose of this study.

EXPERIMENTAL

Two separate studies were made, using four subjects for each study. The subjects were young graduate women of the nutrition department of The University of Chicago. Each study was divided into two 8-day periods. During the first

period, the subjects received a basal diet only. In the second period the basal diet was supplemented with 1000 cc. orange juice daily for one study, and for the other study, two of the subjects received 1000 cc. orange juice and two of them 1000 cc. grape juice daily. The subjects were kept on the basal diet for 3 days before collections and analyses were begun, and, likewise, a 3-day preliminary or adjustment period was allowed when the orange juice and grape juice additions were made. The analyses included determination of pH, titratable acidity, organic acids as a whole and citric acid.

The basal diet in the first study, which we shall designate diet A, consisted of the following: Apple sauce, 100 gm.; canned tomatoes, 100 gm.; thin cream, 5 tbs.; lean beef, 90 gm.; eggs, 90 gm.; head lettuce, 50 gm.; Irish potatoes, 150 gm.; cornflakes, 20 gm.; whole milk, $1\frac{1}{4}$ cups; canned peaches, 100 gm.; coffee, 1 to 2 cups; bread, butter, vanilla wafers, and salad oil in amounts to complete the caloric requirements of the different subjects. Diet B, which constituted the basal diet of the second study, differed in the following respects from diet A. The meat was reduced to 75 gm.; thick cream, $\frac{1}{2}$ cup, replaced the thin cream; the milk was omitted and shredded wheat biscuits (27 gm.) were used instead of cornflakes. Calculation of the potential acidity and alkalinity of the basal diets showed slight excess acidity (table 1). The addition of the fruit juices rendered the diets distinctly alkaline.

The caloric needs of all of the subjects while on the basal diet were evidently met, as was shown by the fact that weights remained constant, and in some instances the subjects showed small gains. The difference in caloric value between the basal diet and the basal diet with the fruit juice additions was, therefore, considered to be of no consequence in this study.

All food was weighed, prepared, and served in the laboratory, the subjects reporting for meals at 7:30, 12:30 and 6:30 each day. The orange juice was prepared from California oranges with an electric squeezer, so that it included a large part of the crushed pulp. The grape juice was a well-known commercial brand.

The collections of urine were made in clean wide mouth bottles with a sufficient layer of toluene to protect the urine from the air. At the end of the 24-hour period the specimens of each subject were thoroughly mixed and the volume recorded. The pH and titratable acidity were determined at once and organic acids within a few hours. The citric acid determination was begun within 24 hours, but the method involved steps requiring several days for completion.

TABLE 1

Acid base values of basal diets and fruit juice supplements (calculated from Sherman's figures)

FOOD	WEIGHT, GRAMS	APPROXIMATE POTENTIAL ACIDITY, CUBIC CENTIMETERS N ACID	APPROXIMATE POTENTIAL ALKALINITY, CUBIC CENTIMETERS N BASE
Apple sauce	100		2.0
Canned tomatoes	100		3.0
Lettuce	50		3.7
Potatoes	150		10.5
Canned peaches	100		3.0
Milk	300-0		7.11- 0
Lean beef	90-75	10.8- 9	
Eggs	90	9.9	
Cornflakes	20-0	2.0- 0	
Shredded wheat	0-27	0 - 3.3	
Bread (white)	125 (average)	8.0	
Vanilla wafers	40 (average)	2.5	
	Totals	33.2-32.7	29.3-22.4
Orange juice	928		41.66
Grape juice	800		31.2

Note. Where two figures are given those in the first column apply to diet A and those in the second column to diet B only. Calculations for butter, salad oil, and cream are omitted because figures for these could not be obtained.

The pH determinations were made colorimetrically, great care being taken to protect the samples from the air by covering with mineral oil, after carefully removing with a pipette from beneath the layer of toluene, with which the urine was kept covered at all times. Folin's method ('22) was used for the determination of titratable acidity; organic acids were titrated according to the method of Van Slyke and Palmer

('20) and, for citric acid, the pentabromacetone method, as modified by Amberg and McClure ('17) and McClure ('22), was employed. The results are given in tables 2 and 3.

The changes in urinary pH, titratable acidity and total organic acids produced by the orange juice and grape juice agree, in general, with results obtained by other workers.

There is a significant increase in citric acid excretion with both the orange juice and the grape juice. This increase is slightly greater with the orange juice, but the ratio of the amount excreted to the amount ingested is very much higher for the grape juice. With one subject, the increased excretion resulting from the grape juice actually exceeds by 20 per cent the amount of citric acid contained in the grape juice. It is evident from these results that citric acid must have a source other than that of the citric acid ingested.

The relation between the citric acid excreted and the total organic acids excreted is also of interest. There is no constancy in this relationship either on the basal diets or on the basal diets with the fruit juice supplements.

If the average citric acid increase for each subject for the 5-day period is compared with the average total organic acid increase produced by the orange juice, the former is approximately 40 to 60 per cent of the latter. With the grape juice, the citric acid increase represents 35 to 40 per cent of the total organic acid increase. It is evident, then, that organic acids other than citric account for a considerable portion of the increased organic acid output resulting from the ingestion of orange juice. Since the acid of orange juice appears (Hartman and Hillig, '30) to be practically 100 per cent citric acid the other acids must be formed in the utilization of the citric acid or have some other metabolic source. The increase in citric acid as compared with the increase in total organic acids produced by the grape juice is far too high to be explained on the basis of unutilized citric acid of the grape juice. It can only be accounted for as representing chiefly a metabolic product.

TABLE 2

Effect of orange juice on acid components of the urine

	DAY	VOLUME, CUBIC CENTI- METERS	pH	0.1 N TITRATABLE ACIDITY, CUBIC CENTI- METERS	OITRIG ACID		0.1 N ORGANIC ACIDS CUBIC CENTI- METERS	pH	0.1 N TITRATABLE ACIDITY, CUBIC CENTI- METERS	OITRIG ACID			
					Grams	Cubic centimeters 0.1 N acid				Grams	Cubic centimeters 0.1 N acid		
Basal diet A	Subject B.												
	1	Adjustment period—no analyses.											
	3												
	4	1390	5.8	264	556	5.9	280	717		
	5	1200	5.5	375	489	0.403	63	6.0	269	806	0.664		
	6	900	5.6	278	413	0.307	48	5.7	287	496	0.427		
	7	790	6.0	224	349	0.426	66	5.5	285	416	0.510		
	8	420	5.4	325	368	0.310	48	5.4	323	406	0.474		
		Aver		5.6	293	435	0.361	56	5.7	288	568	0.527	
Basal diet A plus 1000 cc. orange juice	Subject B.												
	1	Adjustment period—no analyses.											
	3												
	4	1940	6.5	148	626	0.853	133	6.1	251	692		
	5	1700	6.8	147	487	1.044	163	6.5	215	685	2.102		
	6	1640	6.8	153	681	1.017	159	6.6	185	849	1.746		
	7	1500	6.8	137	585	0.925	144	6.0	201	699	1.749		
	8	1410	6.8	147	663	0.937	146	6.3	234	907	1.688		
		Aver		6.7	146	608	0.962	150	6.3	217	746	1.821	
Basal diet B	Subject R.												
	1	Adjustment period—no analyses.											
	3												
	4	1500	5.4	240	444	0.463	72	5.5	262	469	0.424		
	5	1635	5.4	304	444	0.478	74	5.9	238	474	0.570		
	6	2350	5.5	363	605	0.796	124	5.6	218	418	0.489		
	7	1650	5.4	308	505	0.702	109	5.6	298	408	0.325		
	8	1825	6.3	198	563	0.815	127						
		Aver.		5.6	282	512	0.658	102	5.6	254	442	0.452	

Basal diet B plus 1000 cc. orange juice	Adjustment period—no analyses.			Adjustment period—no analyses.					
	1	2	3	4	5	6	7	8	Aver.
	1830	2000	1590	1605	1720	6.7	6.5	6.3	6.7
	190	138	240	171	241	6.0	6.4	6.0	6.4
	609	674	604	614	626	0.929	1.242	1.381	1.068
	145	194	216	167	152	0.975	1.115	0.975	1.115
	174								
	Aver.								
	1395	1420	1900	1519	Aver.	6.6	6.6	6.3	6.6
	124	186	194	159		649	513	518	475
	0.552	0.681	0.895	0.617					
	86	106	140	96					
	107								
	Subject C.S.								
Basal diet A	Adjustment period—no analyses.			Adjustment period—no analyses.					
	1	2	3	4	5	6	7	8	Aver.
	2050	1350	1350	1560	720	6.2	6.0	5.8	5.6
	232	283	253	306	267	5.8	5.4	5.4	5.8
	508	489	540	507	430	0.648	0.451	0.541	0.748
	...	101	70	84	115
	93								
	Aver.								
	1600	950	740	590	420	5.8	5.8	5.6	5.6
	209	219	269	298	212	512	478	482	424

	75								
	Aver.								
	1920	2010	2360	1780	1700	6.9	6.8	6.8	6.8
	125	178	144	139	170	660	503	566	552
	0.806	0.924	0.944	0.925	0.816				
	126	144	147	144	127				
	138								
	Aver.								
	1920	2010	2360	1780	1700	6.9	6.8	6.8	6.8
	125	178	144	139	170	660	503	566	552
	0.806	0.924	0.944	0.925	0.816				
	126	144	147	144	127				
	138								
	Aver.								
	1920	2010	2360	1780	1700	6.9	6.8	6.8	6.8
	125	178	144	139	170	660	503	566	552
	0.806	0.924	0.944	0.925	0.816				
	126	144	147	144	127				
	138								
	Aver.								
	1920	2010	2360	1780	1700	6.9	6.8	6.8	6.8
	125	178	144	139	170	660	503	566	552
	0.806	0.924	0.944	0.925	0.816				
	126	144	147	144	127				
	138								
	Aver.								

TABLE 8

Effect of grape juice on acid components of urine

	DAY	VOLUME, CUBIC CENTI- METERS	pH	0 1 N TITRATABLE ACIDITY, CUBIC CENTI- METERS	0 1 N ORGANIC ACIDS CUBIC CENTI- METERS	CITRIC ACID		VOLUME, CUBIC CENTI- METERS	pH	0 1 N TITRATABLE ACIDITY, CUBIC CENTI- METERS	0 1 N ORGANIC ACIDS CUBIC CENTI- METERS	CITRIC ACID	
						Grams	Cubic centimeters 0 1 N acid					Grams	Cubic centimeters 0 1 N acid
Subject C.													
Basal diet B	1	Adjustment period—no analyses.											
	2												
	3												
	4	1170	5.5	323	531	0.721	112	5.4	246	454	0.623	97	
	5	1790	5.5	294	494	0.755	121	6.4	166	450	0.584	91	
	6	1780	5.5	332	536	0.930	145	6.1	201	453	0.671	105	
	7	1695	5.9	282	528	1.033	161	5.9	216	424	0.546	85	
	8	1690	6.1	210	538	0.917	143	6.3	160	452	0.748	117	
		Aver.		5.7	288	525	0.871	134	6.0	197	446	0.634	99
	Adjustment period—no analyses.												
Basal diet B plus grape juice	1	Adjustment period—no analyses.											
	2												
	3												
	4	1699	5.6	320	552	0.982	137	6.8	175	556	1.001	156	
	5	1820	6.3	202	621	1.164	182	6.1	195	573	0.900	140	
	6	1740	5.8	270	576	1.083	170	6.1	207	524	0.866	135	
	7	1665	6.3	218	611	0.980	153	6.3	206	540	0.895	141	
	8	1770	6.0	236	618	0.934	146	6.3	196	548	0.886	138	
		Aver.		6.0	258	595	1.019	159	6.3	195	548	0.909	141
	Adjustment period—no analyses.												

Note: The figures for pH titratable acidity and organic acids for subjects R., K., C. and H. are the results of analyses made by students in a problem course. They are used by courtesy of Mrs. Ruth Cowan Clouse, who conducted the course.

TABLE 4

Citric acid ingested and excreted as a result of adding orange juice and grape juice to the basal diet

SUBJECT	B	S.	M. H.	O. S.	K	R.	C	H.
Average citric acid excretion on basal diet. Grams	0.361	0.527	0.597	0.482	0.452	0.658	0.871	0.634
Average citric acid excretion on basal diet + 1000 cc. orange juice. Grams	0.962	1.821	0.956	0.883	0.686	1.115		
Average citric acid excretion on basal diet + 1000 cc. grape juice. Grams							1.019	0.909
Average increase in citric acid excretion. Grams	0.601	1.294	0.359	0.401	0.234	0.457	0.148	0.275
Amount of citric acid ingested in 1000 cc. orange juice. Grams	7.95	7.95	7.95	7.95	8.51	8.51		
Amount of citric acid ingested in 1000 cc. grape juice. Grams							0.23 ¹	0.23 ¹
Increased excretion in terms of per cent of amount ingested	7.5	16.6	4.5	5.3	2.6	5.3	64	120

¹ Based on average figures reported in J. Assoc. Official Agr. Chem., 1930, 13, 99.

A very small part of the increase in total organic acids with both the orange juice and grape juice is due to uric acid according to the findings of Clouse previously cited. Determination of other acids concerned in this increase must await the development of new methods now being investigated for use in the study of urinary acidity.

SUMMARY

Two studies made with eight women subjects on the effect of orange juice and grape juice on the urinary excretion of citric acid are reported. The effect on urinary pH, titratable acidity, and excretion of total organic acids was also observed.

The following results were obtained when 1000 cc. orange juice or 1000 cc. grape juice were added to a basal diet.

1. The pH was increased and the titratable acidity was decreased. Organic acid excretion as a whole was increased. These results confirm those obtained by other workers.

2. Citric acid excretion was increased. The increase produced by the orange juice was slightly greater than that produced by the grape juice, but the ratio of the amount of citric acid excreted to the amount ingested was very much higher for the grape juice. With one subject, the increase represented 20 per cent more than the citric acid contained in the grape juice. The latter result indicates a metabolic source of citric acid.

3. The increase in citric acid with the orange juice represented only 40 to 60 per cent of the total organic acid increase. Other organic acids must have been formed in the body.

4. Thirty-five to 40 per cent of the total organic acid increase with the grape juice was due to citric acid. Only a very small part of this could have come from the citric acid of the grape juice, and therefore, most of it can be accounted for only on the basis of having a metabolic origin.

The organic acids representing products of metabolism may arise as a result of the alkalizing effect of the fruit juices.

The author wishes to express her appreciation of the interest and advice of Mrs. Ruth Cowan Clouse at whose suggestion this study was begun. The hearty cooperation of the young women serving as subjects is gratefully acknowledged.

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URINARY EXCRETION OF CITRIC ACID

II. EFFECT OF INGESTION OF CITRIC ACID, SODIUM CITRATE AND SODIUM BICARBONATE

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In a previous paper (Schuck, '34) the increased output of citric acid when orange juice and grape juice supplements were added to a basal diet was reported. As a result of this study it was decided to investigate the influence of pure crystalline citric acid, sodium citrate and sodium bicarbonate upon citric acid excretion in order to obtain further information on: 1) The effect of an increased citric acid intake. 2) The effect of increased alkalinity together with increased citrate ion ingestion. 3) The effect of merely increasing alkalinity.

At the time this study was begun there were with the exception of the work of Gonce and Templeton ('30) no published reports on the effect on citric acid excretion of feeding citric acid to humans. And so far as the author was able to determine, the effect of sodium citrate on citric acid excretion had not previously been studied with humans.

Gonce and Templeton ('30) investigated the citric acid excretion of four normal children 7 to 12 years of age. They fed a basal diet for 2 days, and then supplemented the basal diet with dehydrated citric acid for a period of 3 days. The citric acid was fed at a level of 4 gm. per 15 pounds of body weight, with a range of 11 to 13 gm. daily for the different children. They found that the citric acid supplement did not result in increased citric acid excretion.

A number of studies have been reported on the effect of the administration of sodium bicarbonate on total organic acid excretion and on the excretion of certain specific organic acids, including citric. Fanconi ('28) observed that the organic acid excretion on a vegetable diet, which was already high, could be further increased by the addition of sodium bicarbonate or the potassium salt of another organic acid. Goiffon ('25, '28) observed increased excretion of citric acid on doses of sodium bicarbonate of from 5 to 15 gm. McCleod and Knapp ('18) found that lactic acid output was increased by the ingestion of sodium bicarbonate. Fasold ('30) in a study on a 10-year-old boy, in which he determined total organic acids, volatile acids, hippuric and citric acid, found that the total organic acids, hippuric and citric acid, were all higher on a vegetable than on a meat diet. When he further increased the alkalinity of the urine by adding sodium bicarbonate to the vegetable diet, the latter constituents were further increased. Östberg ('30) at about the same time, reported increased citric acid elimination resulting from administration of sodium bicarbonate. He found that there was a close correlation between citric acid excretion and ammonia excretion. With a decrease in citric acid, there was an increase in ammonia, and vice versa. As a result, he concluded that citric acid plays at least a small part in acid-base balance regulation.

In a later study Östberg ('31) reports an investigation of the effect of citric acid, sodium citrate, sodium bicarbonate, hydrochloric acid, ammonium chloride, calcium chloride, and a low carbohydrate to fat ratio on citric acid excretion. He found that with citric acid doses of from 10 to 15 gm., there was greatly increased output in one case, and in another case a decrease. Thirty grams showed in one case an increase from 0.78 to 1.5 gm., and in another only a small increase. Thirty to 40 gm. gave for one subject in periods of 2 or 3 days a slightly decreased output, accompanied by a decrease in pH and increase in ammonia. This variability in effect led to the conclusion that in cases in which citric acid influences

the acid-base balance, its administration brings about a decreased output, and in cases where there is no effect on the acid-base balance, administration of citric acid may increase its excretion. Östberg ('31) found that increased alkalinity due to ingestion of either sodium citrate or sodium bicarbonate gave increased excretion of citric acid.

Boothby and Adams ('32) have confirmed Östberg's results with sodium bicarbonate, but they feel that the significance of citric acid as a factor in acid-base balance regulation is still questionable. They point out in this connection that it is possible to have an acid urine containing large amounts of organic acids with the normal amount of citric acid. Sullman and Shearer ('32) obtained an increase in citric acid excretion with both sodium carbonate and sodium citrate, but when both were administered at the same time the figure was not significantly different from that obtained when either was used alone.

EXPERIMENTAL

The subjects for this study were six young graduate women of the nutrition department of The University of Chicago. Four of these, B., M.H., S. and C.S., were the same as those serving in the study previously reported (Schuck, '34), and the basal diet for this group was diet A, the composition of which has already been given. The basal diet for the two new subjects, M. and V., which we shall designate diet C, consisted of the following: apple sauce, 100 gm.; cornflakes, 20 gm.; American cheese, 30 gm.; head lettuce, 100 gm.; canned tomatoes, 100 gm.; potatoes, 150 gm.; ground lean steak, 90 gm.; canned peaches, 100 gm.; whole milk, $1\frac{1}{4}$ cups; thin cream, $\frac{1}{4}$ cup; bread, butter and vanilla wafers in amounts to complete the caloric requirements of the different subjects.

The weights of the subjects were followed as before, and their constancy indicated that caloric needs were met throughout the experiment.

The supplements used were citric acids, 12 gm. daily, a chemically equivalent amount of sodium citrate, and sodium

bicarbonate, 10 gm. daily. The citric acid was administered to all six subjects, the sodium citrate to B., M.H., S. and C.S., and the sodium bicarbonate to C.S., M. and V. An 8-day period was allowed for each supplement, the first 3 serving as preliminary days, during which the subject might become adjusted to the new substance. Collections and analyses were made during the remaining 5 days of the period.

The supplements were dissolved in water and the daily amounts were consumed in three separate doses through the day. The citric acid was diluted and sweetened to make a palatable and pleasing beverage, but palatability could not be attained with the other supplements.

Urine collections and analyses were made as described in an earlier study (Schuck, '34). The results are given in tables 1 and 2.

It is apparent that the ingestion of citric acid had little or no effect on the pH of the urine. In most cases, there was a slight decrease in titratable acidity and a decrease in the total organic acid excretion. The citric acid output was decreased in some instances and increased in others. As can readily be seen, there was no parallelism between the total organic acid excretion, as measured by the Van Slyke Palmer method, and the citric acid excretion—a finding which is in agreement with observations made by Östberg ('31).

The sodium citrate gave in every case a marked increase in pH, a marked decrease in titratable acidity, and a large increase in the total organic acids and citric acid excretion. The total organic acids and citric acid amounted to two or three times the excretion on the basal diet. The effect on pH and citric acid excretion produced by the sodium citrate is in general agreement with the results obtained by Östberg ('31). He fed sodium citrate at lower levels, so that the change in pH was not so great, and correspondingly the increase in citric acid excretion was less.

The effect of the sodium bicarbonate on the pH, titratable acidity and citric acid was similar to that of the sodium citrate, in that there was a significant increase in the pH and

TABLE 1

Effect of citric acid and sodium citrate on acid components of the urine

	DAY	VOLUME, CUBIC CENTI- METERS	pH	0.1 N TITRATABLE ACIDITY, CUBIC CENTI- METERS	CITRIC ACID		0.1 N ORGANIC ACIDS CUBIC CENTI- METERS	pH	0.1 N TITRATABLE ACIDITY, CUBIC CENTI- METERS	0.1 N ORGANIC ACIDS CUBIC CENTI- METERS	CITRIC ACID	
					Grams	Cubic centimeters					Grams	Cubic centimeters
Basal diet A	Subject B.											
	1	Adjustment period—no analyses.										
	2											
	3											
	4	1390	5.8	264	556	6.2	232	508
	5	1200	5.5	375	489	0.403	63	6.0	285	489	0.648	101
	6	900	5.6	278	413	0.307	48	5.8	253	540	0.451	70
	7	790	6.0	224	349	0.426	65	5.6	306	507	0.541	84
	8	420	5.4	325	368	0.310	48	5.4	267	430	0.748	117
		Aver.		5.6	293	435	0.361	56	5.8	268	495	0.597
Basal diet + citric acid	Subject M.H.											
	1	Adjustment period—no analyses.										
	2											
	3											
	4	1190	5.9	140	339	0.547	84	6.0	242	443	0.576	90
	5	960	6.0	230	345	0.555	87	6.3	189	467	0.481	75
	6	770	5.6	263	385	0.622	97	6.5	86	470	0.462	72
	7	680	5.4	296	467	0.598	93	5.7	224	492	0.481	75
	8	560	5.7	192	348	0.560	87	6.2	184	501	0.512	80
		Aver.		5.7	244	376	0.576	90	6.1	181	474	0.502
Basal diet + Na citrate	Subject M.H.											
	1	Adjustment period—no analyses.										
	2											
	3											
	4	950	8.3	41	1001	1.243	194	8.2	21	682	1.058	165
	5	1280	8.4	45	1303	1.321	206	8.5	44	1365	1.418	221
	6	1550	8.3	121	867	8.4	125	1274	1.507	250
	7	1560	8.2	162	1009	1.094	171	8.3	214	1567	1.501	234
	8	1540	8.2	194	1134	1.212	189	8.2	150	1867	1.984	325
		Aver.		8.2	112	1262	1.217	190	8.3	110	1357	1.489

TABLE 2

Effect of citric acid, sodium citrate and sodium bicarbonate on acid components of the urine

	DAY	VOLUME, CUBIC CENTI- METERS	pH	0.1 N TITRATABLE ACIDITY, CUBIC CENTI- METERS	0.1 N ORGANIC ACIDS CUBIC CENTI- METERS	CITRIC ACID		Subject S.	0.1 N TITRATABLE ACIDITY, CUBIC CENTI- METERS	0.1 N ORGANIC ACIDS CUBIC CENTI- METERS	CITRIC ACID		
						Grams	Cubic centimeters				Grams	Cubic centimeters	
Basal diet A	1	Adjustment period—no analyses.										Subject OS.	
	2											Adjustment period—no analyses.	
	3												
	4	1380	5.9	280	717	1600	5.9	209	512	...	
	5	1400	6.0	269	806	0.664	104	950	5.8	219	478	0.437	
	6	1080	5.7	287	496	0.427	67	740	5.6	269	482	0.472	
	7	690	5.5	285	416	0.510	79	590	5.6	298	424	
	8	515	5.4	326	406	0.474	74	420	5.6	212	436	0.537	
		Aver.	5.7	288	568	0.527	82	Aver.	5.7	241	446	0.482	
		Adjustment period—no analyses.											
Basal diet + citric acid	1	Adjustment period—no analyses.										Adjustment period—no analyses.	
	2												
	3												
	4	2000	5.7	288	400	0.760	119	2150	6.1	187	458	0.871	
	5	1900	5.6	306	474	0.836	130	1460	5.9	213	420	0.753	
	6	1340	5.6	344	573	1.018	160	1950	5.7	244	421	0.818	
	7	1980	5.7	292	583	1.029	161	1180	5.7	226	500	0.778	
	8	2300	5.4	361	753	1.204	188	1080	5.7	225	401	0.864	
		Aver.	5.6	318	556	0.969	151	Aver.	5.8	219	440	0.806	
		Adjustment period—no analyses.											
Basal diet + Na citrate	1	Adjustment period—no analyses.										Adjustment period—no analyses.	
	2												
	3												
	4	2070	8.1	62	1275	2.260	353	1380	8.2	18	1137	1.460	
	5	1450	8.3	55	1352	2.146	335	1050	8.4	32	1827	1.507	
	6	2400	8.2	190	1017	2.640	412	1990	8.2	74	812	1.576	
	7	2120	8.0	177	768	1.888	295	1760	8.2	188	999	1.535	
	8	2300	8.1	240	1612	2.208	345	1470	8.3	205	1764	1.793	
		Aver.	8.1	140	1204	2.424	379	Aver.	8.3	103	1307	1.574	
		Adjustment period—no analyses.											

		Adjustment period—no analyses.								Adjustment period—no analyses.									
		960	7.8	106	1034	1.234	193			960	7.8	106	1034	1.234	193				
		1850	7.8	103	576	1.461	226			1850	7.8	103	576	1.461	226				
		2340	7.8	111	376	1.380	215			2340	7.8	111	376	1.380	215				
		1250	8.2	20	270	0.767	120			1250	8.2	20	270	0.767	120				
		1810	8.2	65	660	1.309	204			1810	8.2	65	660	1.309	204				
		Aver.	7.9	81	581	1.230	192			Aver.	7.9	81	581	1.230	192				
Subject V.																			
		Adjustment period—no analyses.										Adjustment period—no analyses.							
		3810	6.4	217	504	0.521	81			3810	6.4	217	504	0.521	81				
		3200	6.0	293	450	0.732	114			3200	6.0	293	450	0.732	114				
		4260	6.6	195	494	0.592	92			4260	6.6	195	494	0.592	92				
		4130	6.4	199	582	1.110	173			4130	6.4	199	582	1.110	173				
		4130	6.4	183	560	0.689	107			4130	6.4	183	560	0.689	107				
		Aver.	6.3	217	518	0.728	114			Aver.	6.3	217	518	0.728	114				
Adjustment period—no analyses.																			
		3860	5.6	252	248	0.984	153			3860	5.6	252	248	0.984	153				
		4880	6.4	191	358	0.685	107			4880	6.4	191	358	0.685	107				
		5860	6.4	185	578	0.808	126			5860	6.4	185	578	0.808	126				
		4810	6.2	268	530	0.550	86			4810	6.2	268	530	0.550	86				
		4200	6.5	184	556	0.835	130			4200	6.5	184	556	0.835	130				
		Aver.	6.2	216	454	0.772	120			Aver.	6.2	216	454	0.772	120				
Adjustment period—no analyses.																			
		6510	7.3	155	484	0.856	133			6510	7.3	155	484	0.856	133				
		5440	7.4	194	500	1.115	174			5440	7.4	194	500	1.115	174				
		6550	7.4	221	704	1.506	234			6550	7.4	221	704	1.506	234				
		6170	7.4	159	460	0.734	114			6170	7.4	159	460	0.734	114				
		5910	7.4	141	538	1.229	192			5910	7.4	141	538	1.229	192				
		Aver.	7.4	174	537	1.088	177			Aver.	7.4	174	537	1.088	177				

		Adjustment period—no analyses.								Adjustment period—no analyses.							
		132	0.850	416	132	0.850	416	132	0.850	416	132	0.850	416	132	0.850	416	
		129	0.825	494	129	0.825	494	129	0.825	494	129	0.825	494	129	0.825	494	
		146	0.934	478	146	0.934	478	146	0.934	478	146	0.934	478	146	0.934	478	
		111	0.733	374	111	0.733	374	111	0.733	374	111	0.733	374	111	0.733	374	
		167	1.072	366	167	1.072	366	167	1.072	366	167	1.072	366	167	1.072	366	
		Aver.	0.882	425	136	0.882	425	Aver.	0.882	425	136	0.882	425	Aver.	0.882	425	
Adjustment period—no analyses.																	
		1680	7.3	93	376	1.065	166			1680	7.3	93	376	1.065	166		
		2230	7.3	124	536	1.361	213			2230	7.3	124	536	1.361	213		
		1310	7.3	85	314	0.750	117			1310	7.3	85	314	0.750	117		
		1785	7.5	121	620	1.159	181			1785	7.5	121	620	1.159	181		
		2030	7.5	93	588	1.193	186			2030	7.5	93	588	1.193	186		
		Aver.	7.4	103	487	1.105	173			Aver.	7.4	103	487	1.105	173		

		Adjustment period—no analyses.								Adjustment period—no analyses.							
		1680	7.3	93	376	1.065	166			1680	7.3	93	376	1.065	166		
		2230	7.3	124	536	1.361	213			2230	7.3	124	536	1.361	213		
		1310	7.3	85	314	0.750	117			1310	7.3	85	314	0.750	117		
		1785	7.5	121	620	1.159	181			1785	7.5	121	620	1.159	181		
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citric acid excretion and a decrease in titratable acidity. The excretion of total organic acids did not show a significant change with two of the subjects. One subject, however, showed an appreciable increase in total organic acid excretion. This is in agreement with the observations of other workers (Fanconi, '28; Goiffon, '25; and Fasold, '30).

The difference in effect upon citric acid excretions of feeding citric acid and sodium citrate in chemically equivalent amounts might be explained on the ground that the former is more completely utilized by the body, and that therefore there exists much less likelihood of any of it being excreted when fed in large amounts. Attention should be called to the fact, as having some possible bearing on the question, that the subjects while on the sodium citrate supplement experienced a feeling of nausea, malaise, and in some instances there was a marked diarrhea. The fact, however, that there was little change in the pH of the urine with the administration of citric acid, but that with the sodium citrate there was a marked increase in pH is significant in light of the theory that increased alkalinity is accompanied by increased citric acid excretion. The increase in citric acid output produced by administration of sodium bicarbonate is further evidence for the belief that citric acid is, at least, one of the important organic acids concerned with the elimination of excess base. The smaller sodium bicarbonate dosage used in this study did not bring about as great an increase in pH as was obtained with the sodium citrate, and there was also a smaller increase in the citric acid output. The pH and the citric acid increase, however, closely approach that obtained with the sodium citrate, while the effect upon total organic acid output is considerably less. The meaning of this is not clear.

The results of this investigation give increased evidence for the theory that citric acid excretion is influenced principally by changes in alkalinity, and not by the citric acid intake. They appear to strengthen the view that citric acid is one of the organic acids functioning in acid-base balance regulation.

SUMMARY

A study made with six women subjects of the effect of citric acid, sodium citrate and sodium bicarbonate on the urinary excretions of citric acid is here reported. The effect on urinary pH, titratable acidity, and excretion of total organic acids was also observed.

The following results were obtained with chemically equivalent amounts of citric acid and sodium citrate:

1. The citric acid produced little or no change in the pH of the urine; the sodium citrate brought about a marked increase.

2. The titratable acidity was slightly decreased as a result of the ingestion of citric acid, while the sodium citrate brought about a marked decrease.

3. Total organic acid excretion was decreased by the citric acid, but greatly increased by the sodium citrate.

4. Citric acid excretion was decreased in some cases and increased in others as a result of the citric acid ingestion while the sodium citrate brought about a marked increase in every case. The results here are in agreement with those obtained by Östberg.

5. The total organic acids and citric acid excreted as a result of the ingestion of sodium citrate amounted to two to three times the excretion on the basal diet.

The increase in pH and the decrease in titratable acidity brought about when sodium bicarbonate was fed were accompanied by a small increase in total organic acids and a considerable increase in citric acid excretion. Increase in total organic acid excretion on ingestion of sodium bicarbonate has been noted in the past, and, more recently, like observations have been made on citric acid excretion.

Apparently, citric acid excretion is not dependent upon citric acid ingestion.

Increased evidence is presented here for the view that citric acid is one of the organic acids which plays a part in acid-base balance regulation.

The author wishes to acknowledge with gratitude the hearty co-operation of the young women serving as subjects.

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PROCEEDINGS OF THE FIRST ANNUAL MEETING OF THE AMERICAN INSTITUTE OF NUTRITION

NEW YORK HOSPITAL—CORNELL MEDICAL COLLEGE ASSOCIATION,
NEW YORK CITY, MARCH 28, 1934

MINUTES OF MEETING OF AMERICAN INSTITUTE OF NUTRITION,
NEW YORK CITY, MARCH 28, 1934

The first annual meeting of the enlarged American Institute of Nutrition was held in New York City at Cornell Medical Center on March 28, 1934, as per program mailed to members. Seventy members registered as in attendance and 110 guests were registered. As a matter of fact, a considerably larger number of both members and non-members were in attendance but the others did not register.

President Lafayette B. Mendel presided at the scientific and business sessions. The meeting started promptly on time, at 9.30 A.M.; the business session started promptly at 11.30 and closed exactly at 1.00 P.M. The second scientific session also started promptly on time, and closed a little before 5.00 P.M. All the papers listed for reading, except one, were heard, namely paper no. 8 in the afternoon program, by Israel S. Kleiner and Henry Tauber. This was read by title at the authors' request.

A photograph of the meeting was taken at the afternoon session.

The business session was called to order by President Mendel. The minutes of the organization meeting in Cincinnati were read by the secretary and approved.

The president requested the secretary to announce the names of any members who had died during the past year. The secretary announced that only one, so far as he was aware,

had died, namely Dr. Alfred F. Hess of New York City. The members were asked to rise for a moment in token of respect for Doctor Hess.

First in order was the constitution which had been passed upon at a meeting of the committee appointed last year, which was held at Columbia University Faculty House, Tuesday evening, March 27th, Doctors Mendel, Murlin and Macy only being present. In anticipation of the adoption of the constitution the president asked unanimous consent for the appointment of a nominating committee to report nominations for officers and members of the council not later than 12.45. On motion of Dr. W. C. Rose, the president was given authority to name this nominating committee by unanimous vote. The committee named, consisted of Dr. P. E. Howe, chairman; Dr. Helen Parsons, Dr. W. C. Rose, Dr. L. H. Newburgh and Dr. E. M. Nelson.

The proposed constitution and by-laws were then read slowly section by section by the secretary, but at the chairman's request no action was taken on separate sections. Instead, the president requested that the Society approve his proposal that a committee on revision of the constitution be appointed to report next year. The advisability of this plan would be that all members would have an opportunity to study the constitution, after it was printed and placed in their hands, and they could then communicate with members of the committee on constitution, making such proposal for improvement or amendment as they might see fit.

The constitution and by-laws follow:

CONSTITUTION

- 1 The name of the proposed society is the "American Institute of Nutrition."
2. The purposes of the society are to further the extension of the knowledge of nutrition and to facilitate personal contact between investigators in nutrition and closely related fields of interest.
3. The management of the American Institute of Nutrition shall be vested in a council consisting of the President, Vice-President, Secretary, Treasurer and three additional members.

BY-LAWS

Article I—*Membership*

Section 1. *Eligibility for Membership*: Qualified investigators who have independently conducted and published meritorious original investigations in some phase of the chemistry or physiology of nutrition shall be eligible for membership in the Society.

Section 2. *Nomination*: Nominations for membership shall be made and seconded by members of the Society on blanks furnished by the Secretary. Nominations shall be submitted to the Council who shall determine eligibility and make recommendation to the Society at a regular meeting.

Section 3. *Election to Membership*: A. A nominee for membership may be voted for by ballot at any meeting of the Society after the Council has reported its findings on his eligibility. B. A majority of the ballots cast shall elect.

Section 4. *Forfeiture*: If a majority of the Council after due notice to the member in question and opportunity for a hearing, shall decide that the interests of the Society require the expulsion of a member, the Secretary shall send a notice of this decision to each member at least two weeks before the next annual meeting. At this meeting the Secretary shall, on behalf of the Council, propose the expulsion; and if two-thirds of the members present vote for it, the member shall be expelled, his assessment for the current year shall be returned to him, and he shall cease to be a member of the Society.

Article II—*Meetings and Quorum*

Section 1. *Annual*: The annual meeting of the Society shall be held on the date fixed by the Certificate of Incorporation.

Section 2. *Special*: A special meeting may be called at any time by the President, or in case of his absence or disability, by the Vice-President, and must be called at the request in writing of a majority of the Council or fifteen members of the Society. Notice specifying the purpose of such meeting shall be mailed to each member at least ten days previous thereto. The Council shall select the places at which meetings shall be held.

Section 3. *Quorum*: Fifteen members shall constitute a quorum at all meetings of the Society, but in absence of a quorum any number shall be sufficient to adjourn to a fixed date.

Article III—*Officials*

Section 1. *Officers*: The officers shall be a President, a Vice-President, a Secretary, and a Treasurer, who shall be elected annually by the members of the Society. Their terms of office shall commence with the close of the annual meeting at which they are elected.

Section 2. *Council*: The officers so selected and three additional members, one of whom shall be elected at each annual meeting to serve a term of three years, shall constitute a Board of Trustees and shall be known as "The Council." (When this provision is first put into effect one member shall be elected for one year, one for two years and the third for three years.)

Section 3. *Duties of Officers:* The powers and duties of the officers elected by the Society shall be such as usually devolve upon their respective positions.

Article IV—*The Council*

Section 1. *Powers:* The general management of the Society during the intervals between meetings shall be vested in the Council, which shall regularly perform the ordinary duties of an executive committee and possess all the powers conferred upon the Board of Trustees of an educational institution chartered by the Education Department of the University of the State of New York. A provisional charter was issued to the American Institute of Nutrition under date of September 27, 1928.

Section 2. *Reports:* The Council shall report to the Society its findings on the eligibility of candidates for membership, and on all charges of a violation of these By-Laws.

Article V—*Nominating Committee*

Section 1. *Membership:* A. The Nominating Committee shall consist of five members appointed for the coming year by the retiring President. Members who have served on the Nominating Committee for two consecutive years shall be ineligible for reappointment until after a lapse of one year. B. The President shall designate one member to be Chairman of the Nominating Committee.

Section 2. *Nomination of Officials:* A. The Nominating Committee shall make at least one nomination for each of the four offices and for each of the additional positions in the Council to be filled by vote of the members. B. The nominations by the Nominating Committee must be transmitted to the Secretary at least one month before the annual meeting at which they are to be considered. C. The Secretary shall send to every member, at least two weeks before the annual meeting, the list of nominees presented to him by the Nominating Committee and at the same time shall notify all the members that they may vote by proxy or by mail. D. Additional nominations for the officers and for membership in the Council may be made by any member at the opening of the first executive session of any annual meeting.

Section 3. *Election of Officials:* A. The Secretary shall receive and present to the tellers, appointed by the President to take charge of the election, all signed ballots forwarded by absent members. B. All elective officials shall be selected by ballot at the close of the first executive session of each annual meeting. C. A majority of the votes cast shall be necessary to elect an official.

Section 4. *Filling of Vacancies:* A. The Nominating Committee shall fill all vacancies in elective positions except such as may occur at a meeting of the Society. B. The President of the Society shall fill all vacancies in appointive positions.

Article VI—*Financial*

Section 1. *Dues:* Annual assessments shall be determined by majority vote at the annual meetings, upon the recommendation of the Council, and shall be due within a month after the annual meeting.

Section 2. *Expenditures*: No expenditures from the general funds of the Society except those required in the performance of the ordinary official duties shall be made except by vote of the Society or the Council.

Section 3. *Penalty for non-payment of Dues*: *A* Members in arrears for dues for two consecutive years shall forfeit their membership. *B*. Delinquent members may be reinstated by the Council provided all indebtedness to the Society is liquidated.

Article VII—*The Journal of Nutrition*

Section 1. The American Institute of Nutrition designates The Journal of Nutrition as its official organ of publication.

Section 2. In accordance with the expressed wish of The Wistar Institute of Anatomy and Biology, owner and publisher of The Journal of Nutrition, the American Institute of Nutrition shall nominate members of the Editorial Board for its official organ. *A*. Three members of this Institute shall be nominated as members of the Editorial Board each year to serve a term of four years, replacing three retiring members. *B*. Retiring editors shall not be eligible for renomination until one year after their retirement. *C*. The managing editor shall be chosen by the Editorial Board to serve a period of five years

Article VIII—*Papers on Scientific Subjects*

Section 1. The Secretary shall be authorized to arrange programs for the scientific sessions at the annual meetings.

Article IX—*Changes in Constitution and By-Laws*

Section 1. Proposed changes in the Constitution and By-Laws must be sent in writing to the Secretary at least one month before the date of the meeting at which they are to be considered, and must be signed by at least three members. The Secretary shall send a printed copy of any proposed change to each member at least two weeks before the next meeting and shall notify all members that they may vote by proxy.

Section 2. If at this meeting two-thirds of the votes cast shall favor the proposed change, it shall be made.

Adopted
March 28, 1934

LAFAYETTE B. MENDEL, *Chairman*
PAUL E. HOWE
MARY S. ROSE
ICIE G. MACY
JOHN R. MURLIN

After the reading of the constitution, on motion by Doctor Howe the constitution and by-laws were adopted by unanimous vote. As a committee to consider such revisions as might seem to be in order, the president appointed Dr. Paul E. Howe, chairman; Dr. M. S. Rose, Dr. Icie G. Macy, Dr. A. W. Rowe and Dr. J. R. Murlin.

The financial report of the secretary-treasurer next was read and accepted. An auditing committee, consisting of Dr. W. M. Sperry and Dr. A. H. Smith, previously appointed by the president, found the financial statement correct.

The council which had met at Columbia University Faculty House on Tuesday evening May 27th, reported the following names as qualified for membership in the American Institute of Nutrition:

Sponsored by

William H. Adolph	L. B. Mendel	A. H. Smith
Franklin Church Bing	V. C. Meyers	L. B. Mendel
Norman R. Blatherwick	L. B. Mendel	John R. Murlin
John B. Brown	F. A. Hitchcock	J. F. Lyman
David L. Drabkin	D. Wright Wilson	Jas. H. Jones
N. R. Ellis	Paul E. Howe	Edward B. Meigs
Wendell H. Griffith	H. B. Lewis	L. H. Newburgh
Helen A. Hunscher	Icie G. Macy	Lydia J. Roberts
Cornelia Kennedy	Leroy S. Palmer	R. Adams Dutcher
David M. Kydd	John P. Peters	L. B. Mendel
Alvin R. Lamb	Victor E. Nelson	B. H. Thomas
Floyd Heaton Lashmet	L. H. Newburgh	Eugene F. DuBois
Coral A. Lilly	L. H. Newburgh	H. B. Lewis
O. N. H. Long	W. C. Stadie	D. Wright Wilson
P. Mabel Nelson	Pearl P. Swanson	B. H. Thomas
L. C. Norris	W. E. Krauss	Roland M. Bethke
Roe E. Remington	Harold Levine	A. H. Smith
William Thomas Salter	Joseph C. Aub	Alfred T. Shohl
Raymond W. Swift	E. B. Forbes	John R. Murlin
Vincent du Vigneaud	John R. Murlin	Harold B. Pierce

It was moved by Doctor Howe, seconded by Doctor Carpenter, that the secretary cast one ballot in favor of the twenty candidates approved by the council. The motion was carried unanimously, and the secretary declared all elected.

The question of the relation of the American Institute of Nutrition to the Federation of American Societies for Experimental Biology was raised by the president, who stated that in the judgment of the council it would be premature to discuss this matter further at the present meeting. It seemed to him, as to other members of the council, that the present Federation Council was not yet ready to act favorably on the proposal that the institute become a constituent society of the Federation, which was made at the Cincinnati meeting, and in his judgment the American Institute of Nutrition should not be placed in the attitude of supplicants on this proposal. The secretary had been instructed by the council to find out informally whether there was any objection on the part of the Federation officials to the arrangement carried out this year, namely holding the meeting of the American Institute of Nutrition one day in advance of the Federation meeting. (The secretary met with the Federation council in the evening of March 28th and had the explicit assurance from all members of the Federation council, that there was no objection to this arrangement.)

The committee on nomination of officers reported names for the four offices and three members of the council, as provided in the constitution, as follows: President, John R. Murlin; Vice-President, E. F. DuBois; Secretary, Icie G. Macy; Treasurer, W. M. Boothby; members of the council, 1-year term, Agnes Fay Morgan; 2-year term, A. H. Smith; 3-year term, R. M. Bethke. On motion of Dr. Victor Myers and seconded simultaneously by several individuals whose names were not obtained, the secretary was instructed to cast one ballot for the entire slate. The secretary called attention to the fact that this would be embarrassing in view of the fact that his own name appeared on the ballot, and Doctor Mendel therefore volunteered to act as secretary in carrying out the spirit of this motion. He declared all nominees elected.

The dues for 1934-1935 were fixed at \$1.00 by unanimous vote of the Institute.

The question of a permanent charter for the Institute was raised, and the secretary was instructed to place this item on the agenda for the business session next year. Meantime the terms of permanent incorporation were to be investigated by the president-elect.

In accordance with the by-laws just adopted, the chairman named as a nominating committee for 1935 Dr. H. Steenbock, chairman; Dr. E. B. Forbes, Dr. A. W. Rowe, Dr. W. M. Sperry and Dr. Helen Mitchell.

It was announced that the place and date of the next meeting would be determined by the council just elected.

The Institute adjourned at 5.00 o'clock with a rising vote of thanks to the New York Hospital and Cornell Medical Center for the splendid accommodations provided for this meeting, and especially to the local committee for all they had done in the interest of comfort and convenience for the members and their guests.

JOHN R. MURLIN,
Secretary-Treasurer.

PROGRAM OF PAPERS PRESENTED

ABSTRACTS

The absorption and storage of vitamin A by the rat. Carl A. Baumann (by invitation), Blanche M. Rising (by invitation), and Harry Steenbock, Department of Agricultural Chemistry, University of Wisconsin, Madison.

When vitamin A in the form of halibut liver oil was fed to rats the amount of vitamin A stored in the livers was found to parallel the amount of vitamin A in the diet, but only 10 to 20 per cent of the ingested vitamin A, as measured by the SbCl_5 test, was recovered from this organ. Much of the vitamin was destroyed in the digestive tract, but all of the losses could not be attributed to this destruction.

The growth of rats on a low A diet was greater than could be explained by their stores of vitamin A in the liver. Vitamin A reserves were depleted at the rate of 13 blue units a day on a low A diet. The minimum daily dose of vitamin A necessary to produce storage in the liver was 25 blue units. Nevertheless, animals whose liver reserves averaged only 25 blue units grew for 5 weeks and survived for 10 weeks on a vitamin A free diet. Attempts to locate adequate amounts of vitamin A in tissues other than the livers failed.

When equal amounts of halibut oil were fed to normal and to low A rats, the liver stores increased most rapidly in the normal animals. Evidently tissues other than the liver exert a pronounced effect not only upon the storage but upon the depletion of vitamin A reserves.

Disturbance in the water metabolism of albino rats induced by feeding a ration poor in inorganic constituents Pearl P. Swanson and Arthur H. Smith, Nutrition Laboratory of the Foods and Nutrition Department, Iowa State College, Ames, and the Department of Physiological Chemistry, Yale University, New Haven.

Complete withdrawal of inorganic constituents from an otherwise adequate experimental diet causes a disturbance in water metabolism of the albino rat. This is shown by abnormal distribution of water in certain tissues (blood, muscle, kidney, and bone), by retardation of processes of natural dehydration associated with advancing age, and by augmented water intake and urine output.

The adjustment of the experimental animal (37 days old) to the low salt ration is of special significance. After 42 days, a dehydration of muscle occurs, greater than that attributed to advancing age. This condition is reflected in more hydremic blood. After the 90-day experimental period, however, both muscle and blood of these animals contain more water than do the same tissues in normal animals. It is in this latter period that the water intake and the urine output increase markedly.

Associated with dehydration characteristic of the muscle after 42 days are changes in fresh weight of the adrenal gland. During this period, the normal organ increases 35 per cent in weight; the adrenal of the 'low salt animal,' 56 per cent. After this there is a progressive shrinking of the adrenal concomitant with increased and sustained water content of muscle and blood. It appears that certain compensatory changes in size of adrenal occur related to shifts in distribution of water in blood and muscle.

The utilization of energy producing nutriment and protein as affected by individual nutrient deficiencies. I. The effects of cystine deficiency. R. W. Swift (by invitation), O. J. Kahlenberg (by invitation) and E. B. Forbes, Institute of Animal Nutrition, Pennsylvania State College.

Twenty growing rats were fed, by the paired feeding method, in metabolism, growth and body analysis experiments, ten on a cystine deficient diet and ten on the same diet plus cystine. The diets were of the same energy value and were fed in identical quantities to the two rats of each pair.

The effects of cystine deficiency were depression of appetite, growth, and storage of nitrogen and energy; there was increased energy loss as heat, and as urine and feces, but no effect on digestibility of protein.

The relation of the leucines to nutrition. Madelyn Womack (by invitation) and William C. Rose, Laboratory of Physiological Chemistry, University of Illinois, Urbana.

By the use of diets devoid of proteins, but carrying instead synthetic mixtures of highly purified amino acids supplemented with concentrates of our unknown growth essential,¹ it has been demonstrated that both leucine and isoleucine are indispensable dietary components. In the absence of either from the ration young rats cease to grow. On the other hand, the addition of the missing amino acid to the food promptly induces growth.

The relation of norleucine to nutrition is still uncertain. Under conditions similar to those described above, its removal from the food exerts no effect upon body weight. If it is a necessary dietary constituent, the quantities required are extremely small.

¹ Windus, W., Catherwood, F. L., and Rose, W. C., J. Biol. Chem., 94, 173 (1931).

On the relation between the melting point and absorption of fats. L. Emmett Holt, Jr., Herbert C. Tidwell (by invitation) and Sarah Neale (by invitation), Harriet Lane Home of the Johns Hopkins Hospital and the Department of Pediatrics, Johns Hopkins University, Baltimore, Maryland.

The melting point is commonly believed to be an important factor in the absorption of fat, low melting fats being more readily absorbed.

In metabolism experiments on infants we observed the best retention: 1) with fats containing a predominance of shorter chain fatty acids, 2) those with a predominance of unsaturated fatty acids. Both these fats are characterized by low melting points, but it occurred to us that ease of absorption might be due to the peculiarity of chemical constitution rather than to the low melting point. We recently had an opportunity to test this point.

Glycerides of elaidic acid, though isomeric with oleic and containing also a double bond, nevertheless melt at a far higher temperature. By comparing a partially hydrogenated corn oil, rich in elaidin, with a mixture of tripalmitin and tristearin (approximately 2:1) we were able to study two fats of practically identical melting point (50°C.) which differed greatly in their content of unsaturated fatty acid. A striking difference in retention was found. With the

completely saturated tripalmitin-tristearin mixture the average retention was only 61.5 per cent of the intake, whereas with the partially hydrogenated corn oil containing the elaidin 81 to 84 per cent was retained

The results indicate that the chemical constitution of the fat (in this case the presence of double bonds) rather than the melting point is the important factor in fat absorption.

A hitherto undescribed disturbance of calcium metabolism. R. H. Freyberg (by invitation), R. L. Grant (by invitation) and L. H. Newburgh, University of Michigan Medical School, Ann Arbor.

The calcium, phosphorus and energy exchange was studied in a 19-year-old male exhibiting generalized skeletal demineralization, obesity, hypertension and hyposexualism. Serum calcium was high, serum phosphorus low and serum phosphatase high—findings typical of hyperparathyroidism.

When the diet was poor in calcium, the urinary calcium was high, and the calcium balance was markedly negative. When the diet contained abundant calcium, the urinary calcium did not change significantly, the fecal calcium became very high, and the balance remained negative. Intravenously injected calcium was partially excreted in the urine, the remainder retained. When the high calcium diet was supplemented by CaHPO_4 , the urinary calcium was not changed significantly, the fecal calcium was markedly increased, and the balance became only slightly positive. The calcium balance was not significantly changed when viosterol and haliver oil were administered. Administration of NH_4Cl was attended by a marked increase in urinary calcium, no change in fecal calcium, and negative calcium balance. Yeast did not change the urinary calcium; however, the fecal calcium diminished, and the calcium balance became more positive than at any other time. Further study of this yeast effect is now under way.

The phosphorus exchange took the same course as that of the calcium

Undernutrition caused weight loss which followed the prediction for normals.

The effect of the previous diet on the basal respiratory quotient and oxygen consumption. F. A. Hitchcock, Department of Physiology, Ohio State University, Columbus.

The increasing use of the respiratory quotient in studies on the metabolism of the various food stuffs makes desirable a more complete knowledge of the normal variations to which it is subject and of the various factors affecting it. The experiments reported here were carried out to determine what effect, if any, the composition of the diet on the day preceding a basal metabolism test has on the R.Q. and oxygen consumption.

Nine men and two women were used as subjects. Control tests were run on each subject in which there was no attempt made to control the diet. The basal metabolism was then determined after each subject had consumed for one or more days preceding the test, 1) a diet rich in carbohydrates, 2) one rich in fats, and 3) one rich in proteins.

In spite of considerable personal variation the results show that the quotients were appreciably higher after the high carbohydrate diet, while the lowest quotients were obtained after the subject had been on a high fat diet. The high

protein diet also seemed to produce a lowering of the quotient. With a majority of the subjects the lowest oxygen consumption was obtained after the high carbohydrate diet. Analysis of the data shows the extreme susceptibility of the quotient to changes in the ventilation rate. There is also an indication that the factor of fatigue plays an important part in fixing the level of both the R.Q. and the oxygen consumption.

On the measurement of body heat losses. James D. Hardy (introduced by Eugene F. DuBois), Russell Sage Institute of Pathology, in affiliation with the New York Hospital, and the Department of Medicine, Cornell University Medical College, New York.

The development of an accurate method for measuring skin temperature and body radiation has permitted investigations of the heat losses of the body in detail. The radiation losses, under the standard conditions for measurement of the basal metabolic rate in the Sage Calorimeter, have been found to be 55 per cent of the total heat eliminated, convection 20 per cent and vaporization 25 per cent.

Radiation and skin temperature measurements have been made under various atmospheric conditions on normal subjects fully clothed and half clothed. A new method for obtaining the average skin temperature has been developed which weighs the readings according to the surface area of the part examined.

The appearance of cataract and dermatitis in experimental animals given vitamin G deficient diets containing casein and egg albumin. Paul L. Day, William C. Langston (by invitation) and K. W. Cosgrove (by invitation), University of Arkansas School of Medicine, Little Rock.

Comparisons were made between the pathological manifestations produced by vitamin G poor diets containing casein and egg albumin as the sources of protein. Albino rats given a vitamin G deficient diet, containing casein, developed alopecia and cataract, but dermatitis (other than alopecia) appeared only rarely. When dried egg albumin was substituted for the casein, a dermatitis developed, similar to that described by Parsons, but the animals did not show any significant cataractous changes. Chicks given the Ringrose-Norris-Heuser diet E, which contains casein as the source of protein, developed keratitis and cataract, but only mild dermatitis. Chicks given the Ringrose-Norris-Heuser diet F, which contains egg albumin, developed severe dermal lesions, but only slight changes in the cornea and lens. It appears that egg albumin tended to prevent the appearance of cataract, but was a causative factor in the development of dermatitis, in both rats and chicks.

Further investigations concerning the new vitamin B rat growth factor found in whole wheat. Nellie Halliday and Linnea Dennett (by invitation), Michigan Agricultural Experiment Station, Home Economics Section, East Lansing, Michigan.

We have reported the presence in whole wheat of a factor which apparently supplements vitamins B and G for the rat. In its absence growth ceases and certain typical nerve symptoms (very similar to those due to vitamin B₁ deficiency) manifest themselves.

Using a technic which will induce this condition, we have been able to test various materials for their potency in this factor. It is found to be largely concentrated in the outer layers of the grain. It can be extracted by dilute acid or by 50 per cent alcohol, but apparently not by ether. Using bran as the source material, and following in the main the method described by the English workers for vitamin B₄, we have prepared an active concentrate which resembles in many respects crystalline vitamin B₄.

Since the nerve condition can be alleviated and growth be induced by materials which are very low in vitamin B₁ and, since the animals are at all times receiving vitamin G in amounts considered to be adequate, it would seem that the deficiency cannot be due to a lack of either of these two known vitamins, but is probably due to a third factor. This may or may not be vitamin B₄, but there are some characteristics common to both factors.

The vitamin C content of human and guinea pig tissues O. G. King, Chemistry Department, University of Pittsburgh.

Using the titration technic previously described by Bessey and King (J. Biol. Chem., 103, 687), a further study has been made of the rate of depletion of vitamin C from the tissues of guinea pigs with known dietary histories. Depletion follows rapidly and regularly with a scorbutic diet. External indications of the depletion appear much later. The distribution of vitamin C in human tissues is analogous to that in guinea pigs, and has been found to show such marked variations for individuals that a wide zone of depletion appears to be fairly common without external evidence of a deficiency. This intermediate zone may be physiologically significant without being recognized by casual examination.

The effect of high and low cholesterol diets on growth and efficiency of food utilisation in rats. Warren M. Sperry and V. A. Stoyanoff (by invitation), Chemical Laboratory, Babies' Hospital, and the Department of Biological Chemistry, College of Physicians and Surgeons, Columbia University, New York.

Rats were paired at weaning according to sex and in most cases according to litter. One member of each pair was fed a practically cholesterol free, synthetic diet (extracted casein, sugar, Crisco, and supplements); the other received the same diet plus 1 or 2 per cent cholesterol. The rats were weighed at frequent intervals and the total food consumption was determined. The data, which have been analyzed statistically, indicate that the rats receiving cholesterol grew less well and consumed less food than rats receiving no cholesterol. To determine whether the decreased growth was due only to decreased consumption of food, efficiency quotients were calculated according to the formula of Palmer and Kennedy.¹ The results indicate that the cholesterol-fed rats utilized their food less efficiently than rats receiving no cholesterol. The detrimental effect of cholesterol may possibly be due to the fact that it accumulates in large amounts in the liver. In a small series of rats on a natural-food diet, consisting chiefly of grains, no significant differences between rats receiving and not receiving cholesterol were observed.

¹ Palmer, L. S., and Kennedy, C., J. Biol. Chem., 90, 545 (1931). Digestion coefficients were not determined as the diet was the same for each of the groups being compared, except for the cholesterol, for which correction was made.

The influence of cholesterol on the tissues of rats ingesting diets rich and poor in fat. H. H. Williams (by invitation), W. E. Anderson and L. B. Mendel, Department of Physiological Chemistry, Yale University, New Haven.

The rations of all animals beginning at 21 days of age contained 40 per cent of the total calories as dried skimmed milk, the remaining 60 per cent of the energy intake being supplied by 1) cornstarch, 2) coconut oil, or 3) cottonseed oil. Part of the animals in each group received an addition of cholesterol (1 per cent) to their rations. A vitamin supplement of ether extracted yeast and cod liver oil concentrate was also supplied. When the rats had consumed the equivalent of 3000 calories, they were killed and the tissues were examined.

In the high fat diets the addition of cholesterol caused an increase of 20 to 30 per cent in weight of liver when calculated on a body weight basis. This increase was due, at least partly, to the deposition of neutral fat. No difference was obtained when cholesterol was added to the carbohydrate diet which contained approximately 1 per cent fat. Increases in cholesterol content paralleled the total lipid content, the ester fraction exhibiting the greater part of the change. Feeding cholesterol had no effect upon the cholesterol composition of the adrenals; however, these glands were noticeably heavier on the high fat diets. There is some evidence that the cholesterol additions to the high fat diets increased the cholesterol content of the blood plasma, particularly in the ester fraction.

The effect of supplementary iodine on the nutritive value of chick rations.

Arthur D. Holmes, Madeleine G. Pigott (by invitation) and Wendell H. Packard (by invitation), Research Laboratories, The E. L. Patch Company, Boston.

Numerous investigations have supplied data which indicate that the incidence of human goiter varies with the iodine content of the soils of different localities. Less information is available concerning iodine deficiency in animals, but reports for pigs, sheep and other animals indicate that the same situation prevails. Poultry feeds consist largely of cereals and these are quite generally grown in localities reputed to have soils of low iodine content. Accordingly a study has been made to ascertain whether supplementary feeding of iodine enhances the nutritive value of chick rations. Iodine was added at four different levels to a high-grade commercial chick growing ration. The experimental and control rations were fed to five pens of Rhode Island Red chicks from hatching to 12 weeks of age. Data were collected concerning the growth of the chicks, their physical appearance, bone growth, hemoglobin content of blood, the consumption of mash, and the nutritive efficiency of the five mashes under consideration. The results obtained did not indicate that the addition of iodine materially enhanced the nutritive value of the basal ration.

Effects of growth hormone on protein metabolism. N. K. Schaffer (by invitation) and M. O. Lee, Harvard Medical School, Boston.

The administration of anterior pituitary growth hormone to rats over short periods of time was followed by decreases in the concentration of several non-protein nitrogenous constituents of liver, muscle and the whole carcass. The free

amino acids decreased from 5 to 12 per cent and bound (peptide) amino acids decreased from 10 to 24 per cent in liver, muscle and whole carcass. Changes in ammonia were insignificant. Urea plus ammonia decreased from 6 to 21 per cent in the whole carcass, from 20 to 50 per cent in the liver and about 22 per cent in muscle. In hypophysectomized rats there was found a tendency toward higher levels of free amino acids.

The metabolism of fructose. Joseph H. Roe, Department of Biochemistry, School of Medicine, George Washington University, Washington.

A specific colorimetric method for the determination of fructose in blood and urine, based upon the Seliwanoff reaction, has been developed. Proteins and other organic matter are removed with ZnSO_4 and NaOH . Filtrate is mixed with resorcinol, HCl , and alcohol, and warmed at 80°C . in a water bath for 10 minutes. Following its ingestion by rabbits fructose is found in portal blood in considerable quantities and in peripheral blood in much smaller quantities. Analysis of blood samples collected simultaneously from the portal and hepatic veins following fructose ingestion has shown a decrease in fructose and an increase in the total fermentable sugar of the blood which passes through the liver, indicating the liver converts fructose into glucose. After enteral administration of fructose to chloroform poisoned rabbits the blood fructose level remains about the same as in normal animals, but a marked hyperglycemia occurs. This shows that in the damaged liver there is little impairment of the capacity to transform fructose to glucose but marked interference with the glycogenic function of the liver. Data have been obtained which indicate that fructose per se is not affected by insulin, but serves as an indirect physiological antagonist to insulin by its transformation into glucose in passing through the liver.

On the rate of absorption of D-glucose from the intestine of the dog. Harry C. Trimble, Biochemical Laboratory of Harvard Medical School, and Stephen J. Maddock, Laboratory of Surgical Research, Boston City Hospital, Boston. (Introduced by Milton O. Lee.)

In recent communications we have shown (A) that d-glucose is absorbed from the intact gastrointestinal tract of dogs at a rate approximating 1 gm. per kilo per hour,¹ and (B) that to this process the contribution of the stomach is either zero or negligibly small.² In order to test the possibility that the pylorus might prevent the absorption process proceeding at the maximum attainable rate a series of observations have been made upon unanaesthetized animals which received glucose directly into the small intestine. In twelve experiments (1 to 3 hours) the observed rates of absorption averaged 0.92 gm. per kilo per hour. Decreasing the period of observation or increasing the concentration of the administered solution did not yield evidence of higher rates.

¹ J. Biol. Chem., 100, 125, 1933.

² J. Biol. Chem., 103, 285, 1933.

The carbohydrate exchange of the heart of depancreatized dogs. H. E. Himwich and W. Goldfarb (by invitation), Laboratory of Physiology, Yale University School of Medicine, New Haven.

Previous work discloses that not only glucose but also lactic acid^{1, 2, 3} is removed by the heart of normal dogs. The carbohydrate exchange of this organ is therefore normally positive. In the present experiments the carbohydrate exchange of the heart of depancreatized dogs has been studied. Either 48 or 72 hours after aseptic extirpation of the pancreas the animals were anaesthetized with amytal, artificial respiration was instituted and the thorax opened. Blood samples were drawn, practically simultaneously from the coronary sinus and the femoral artery and analyzed for lactic acid and glucose. The combined errors of the method were 6 mg. per cent. The results reveal that in fourteen of sixteen significant observations the carbohydrate exchange is positive. An increased glycogen content of the heart^{4, 5} and a cardiac R.Q. indicating the combustion of fat^{6, 7} suggest that lactic acid and glucose are converted to glycogen chiefly at the expense of the oxidation of fat, the diabetic condition of the animal precluding the adequate oxidation of carbohydrate.

¹ Himwich, H. E., Koskoff, Y. D., and Nahum, L. H. 1928. Proc. Soc. Exp. Biol. Med., 25, 347.

² McGinty, D. A. 1931. Am. J. Physiol., 98, 244.

³ Evans, C. L., DeGraff, A. C., Kosaka, T., Mackenzie, K., Murphy, G. E., Vacek, T., Williams, D. H., Young, F. G. 1933. J. Physiol., 80, 21.

⁴ Cruickshank, E. W. H. 1914. J. Physiol., 47, 1.

⁵ Chambers, W. H., Kennard, M. A., Pollack, H., Dann, M. 1932. J. Biol. Chem., 97, 525.

⁶ Piserico, E. 1925. Arch. Fisiol., 23, 488.

⁷ Cruickshank, E. W. H., and Startup, C. W. 1933. J. Physiol., 80, 179.

READ BY TITLE

The digestion of salivary amylase by trypsin and by papaine. Israel S. Kleiner and Henry Tauber (by invitation), Department of Physiology and Physiological Chemistry, New York Homeopathic Medical College and Flower Hospital, New York.

In studies on the chemical nature of enzymes we have had occasion to test the digestibility of urease, maltase, rennin, emulsin, pepsin and trypsin. With the single exception of emulsin, all of these, under certain conditions, may be digested by proteolytic enzymes, some of them very rapidly. We have now tested salivary amylase and can report that it is slowly inactivated by trypsin and more slowly by H₂S—activated papaine.

A comparison of the antirachitic potency of cod liver oil and irradiated ergosterol on a curative and preventive basis. Walter C. Russell, M. W. Taylor (by invitation) and D. E. Wilcox (by invitation), Department of Agricultural Biochemistry, New Jersey Agricultural Experiment Station, New Brunswick, New Jersey.

When cod liver oil and irradiated ergosterol are compared as to their prevention of leg weakness in the chicken, the amounts fed are expressed in units of vitamin

D determined by a curative method, using the white rat as the assay animal. In the chicken an amount of irradiated ergosterol antirachitically equivalent to cod liver oil, according to a curative rat assay, is not as effective in the prevention of leg weakness as cod liver oil. In order to determine whether the white rat responded equally well to the same number of units of the factor in the form of cod liver oil and irradiated ergosterol, when fed on a preventive basis for 40 days, the two sources of the factor were fed at 0.5, 1, 2, and 10 times the curative (Steenbock) unit per 50 gm. of ration. Practically identical bone ash percentages were obtained at each of these levels. This result is in striking contrast to that observed in the case of the chicken.

Chemical composition and breaking strength of bones of rats consuming a ration extremely poor in inorganic salts. Miriam F. Clarke (by invitation), A. L. Bassin (by invitation) and Arthur H. Smith, Departments of Physiological Chemistry and of Surgery, Yale University, School of Medicine, New Haven.

It has been shown that bone ash is greatly reduced and fragility increased when rats are fed diets containing minimal amounts of inorganic residue. In the present investigation chemical changes occurring in the femur and breaking strength of fibula, radius and humerus were studied. These factors were examined in animals kept on the low salt diet for 3, 6 or 12 weeks, as well as for others treated in a similar fashion but subsequently realimented with adequate diet for varying periods.

Ash, based on weight of dry, extracted femur, was reduced to 44, 42 and 40 per cent after 3, 6 and 12 weeks, respectively, on the low salt diet; values for corresponding age controls were 57, 61 and 64 per cent.

After 3 weeks on the low salt ration, moisture of the femur rose to 57 per cent whereas the control value at this age was 48 per cent.

Realimentation resulted in a return to normal chemical composition when the period on the low salt diet had been short (3 weeks), but a failure to reestablish normal composition when the period was long (12 weeks).

The breaking strength of the bones was reduced to values less than half of those of the controls. Realimentation resulted in a tendency toward over compensation, followed by a relative decrease to values somewhat less than those of the controls.

The possibility of gluconeogenesis from fat IV Non-effect of a high fat meal on the combustion of sugars in human subjects. John R. Murlin, Alan C. Burton (by invitation), Edmund S. Nasset (by invitation) and Sidney Feyder (by invitation), Department of Vital Economics, University of Rochester, Rochester, N. Y.

By means of a semi-automatic respiration calorimeter with closed circuit and the Tissot-Haldane method, the respiratory metabolism and heat production of three subjects subsisting exclusively for 3 days on rich cream were studied. The ketonuria and ketonaemia were followed by analysis for the two fractions diacetic acid + acetone and beta hydroxybutyric acid. Calorimeter periods were introduced on the second and third high fat days; on the former in basal condition, on the latter following a high fat meal. The specific dynamic action therefore

was determined, by simultaneous direct and indirect calorimetry, supplemented by the Tissot-Haldane method. Direct and indirect heat agreed except in one subject, unaccustomed to fat, in whom the R.Q.'s were very low. Approximately 3½ hours after the high fat meal on the third day and next morning (fourth day) without previous food, a large dose of sugar was taken. The assumption was made that if the fat meal had contributed anything to the carbohydrate stores of the body, the sugar would have been oxidized more rapidly than when it was taken post-absorptively. No such effect was obtained. It seems, therefore, the high fat contributed no carbohydrate at this particular time, or for some reason it had not yet become available for combustion.

Studies in lactation. II. Application of criteria of growth in the suckling young to estimation of lactation in the rat. Ray G. Daggs, Department of Vital Economics, University of Rochester, Rochester, N. Y.

Brody's growth measurement method was applied to the growth of suckling young rats. One growth constant was obtained for the period from the fourth to the tenth day of life and another for the period from the tenth to the seventeenth day. The litters were all limited to six young at birth. At about 4 days the habits of the mother become stabilized and on about the sixteenth or seventeenth day the eyes of the young open and they begin to nibble at the mother's food. From a complete analysis of our data on lactation in rats we find in the majority of cases the distinct break at approximately the tenth day that Brody describes. The growth constants differ with a fair degree of significance when the diets of the lactating mothers differ. We believe this method is a more accurate picture of the growth changes in the young due to lactation changes in the mother than is presented by any of the existing means of judging lactation in rats such as percentage weight increase, percentage mortality, etc. It is frankly admitted that growth in suckling young depends upon many more factors than the quantity and quality of the milk supplied. Many of these other factors have been controlled in our experiments.

A study of the relation of diet to dental caries. Martha Koehne and R. W. Bunting (by invitation), in cooperation with Mary Crowley, Philip Jay and Dorothy G. Hard, University of Michigan School of Dentistry, Ann Arbor.

Report is made of the incidence of dental caries in a group of 169 children, 6 to 18 years old, who lived in an orphanage and who were under dental observation for periods varying from 1 to 4½ years. Policies followed in feeding the children for the past 5 or 6 years have been remarkably uniform and results of detailed studies of the food intake of sixteen representative children living under this regime are reported. Quantitative estimations of the concentration of *B. acidophilus* in the saliva of twenty-four children were made at monthly intervals for a period of a year and a half. Correlation is made of dental findings with the quality of food, concentration of *B. acidophilus* in the saliva, carbon dioxide capacity of the saliva, height-weight status of the children, and other factors studied.

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